DEPARTMENT OF THE ARMY
U.S. ARMY INSTITUTE OF PUBLIC HEALTH
5158 BLACKHAWK ROAD
ABERDEEN PROVING GROUND MARYLAND 21010-5403

MCHB-IP-THE

MEMORANDUM FOR Environmental Acquisition and Logistics Sustainment Program (AMSRD-MSF/Mr. Erik Hangeland), U.S. Army Research, Development and Engineering Command, 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424

SUBJECT: Toxicology Study No. 87-XE-0E9V-11, Effects of Oral Guanidinium 3,4-dinitropyrazolate (GDNP) Exposure to Female Rats *(Rattus norvegicus)*, February 2012

1. Five copies of the subject report with Executive Summary are enclosed.

2. Please contact us if this report or any of our services did not meet your expectations.

3. The Army Institute of Public Health point of contact is Dr. Larry Williams, Toxicology Portfolio, Health Effects Research Program. He may be contacted at DSN (312) 584-3980 or commercial (410) 436-3980.

FOR THE DIRECTOR:

CHRIS E. HANSON
COL, VC
Portfolio Director, Toxicology

Encl

USAPHC
U.S. ARMY PUBLIC HEALTH COMMAND
5158 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

Toxicology Study No. 87-XE-0E9V-11, February 2012
Protocol No. 0E9V-30-11-05-01
Toxicology Portfolio

Effects of Oral Guanidinium 3,4- dinitropyrazolate (GDNP)[1] Exposure to Female Rats *(Rattus norvegicus)*

Prepared by Dr. Larry Williams, Health Effects Research Program

Approved for public release; distribution unlimited.

[1]SBIR Data Rights
Contract No.: W911QX-06-C-0138
Contractor Name: Physical Sciences Inc.
Contractor Address: 20 New England Business Center, Andover, MA 01810
Expiration of SBIR Data Rights Period: 30 Sep 2013

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14. ABSTRACT

The Army Environmental Quality Technology Program (EQT) Ordnance Environmental Program (OEP) is dedicated to finding replacements for RDX that will reduce or eliminate the health risks from environmental exposure and will reduce adverse ESOH effects and effects of RDX on readiness and costs associated with training. USARDECOM-sponsored work with Physical Sciences Inc. (Andover, MA) and BAE Ordnance Systems (Kingsport, TN) developed GDNP as a possible replacement for RDX. This document reports the conduct, findings, and conclusions of a progressive series of two oral toxicity studies performed with GDNP in laboratory female rats. The LD50 in female rats in tap water solution is 720 mg/kg. Daily oral exposure to 500 mg/kg-d of GDNP for 14-days causes weight loss, increased liver and spleen organ weights, and adverse histopathologic events in kidney and spleen. These adverse events were not observed in animals receiving lower doses of GDNP. The acute and subacute oral toxicity of GDNP is low and is not limiting to the continued development of GDNP.

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Study Title

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Study Completed

February 2012

Performing Laboratory

Army Institute of Public Health
Toxicology Portfolio
Health Effects Research Program
MCHB-IP-THE
Aberdeen Proving Ground, MD 21010-5403

Laboratory Project ID

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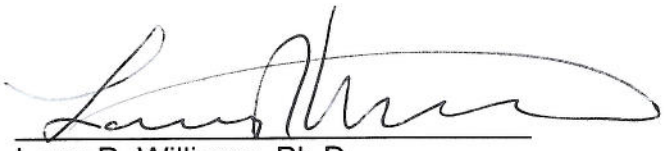

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2-24-12

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Date

Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The test article characterization (purity) was conducted by the manufacturer and it is not known whether the analysis was done in compliance with the above regulation.

No deviations from the aforementioned regulation affected the quality or integrity of the study or the interpretation of the results.

Larry R. Williams, Ph.D.
Study Director
Health Effects Research Program

3-6-12
Date

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February 2012

1 Summary

1.1 Overview

The energetic and toxic properties of guanidinium 3,4- dinitropyrazolate (GDNP) are being determined to support evaluation of GDNP as a possible replacement for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).

1.2 Purpose

The objective of this study was to determine the oral LD_{50}, 95 percent confidence intervals, and slope from oral administration of GDNP, and to determine if adverse effects occur from a 14-day repetitive oral exposure regime of GDNP in the female rat, i.e., derive the no-observable-effect-level (NOAEL) and lowest-observed-effect-level (LOAEL).

Research, development, testing, training, and use of substances potentially less hazardous to human health and the environment is vital to the readiness of the U.S. Army. Safeguarding the health of Soldiers, Civilians, and the environment requires an assessment of alternatives before they are fielded. Continuous assessments begun early in the Research Development Testing and Evaluation (RDT&E) process can save significant time and effort during RDT&E, as well as over the life cycle of the items developed. Residues of pyrotechnics, propellants, explosives, and incendiaries have been found in soil, air, surface, and ground water samples, creating environmental problems and interfering with training activities.

The Army Environmental Quality Technology (EQT) Ordnance Environmental Program (OEP) is dedicated to finding replacements for RDX that will reduce or eliminate the health risks from environmental exposure and will reduce adverse Environment, Safety and Occupational Health (ESOH) effects and effects of RDX on readiness and costs associated with training (USACHPPM 2007b). By identifying unacceptable ESOH effects early in the acquisition process, unacceptable replacements can be identified and unnecessary budget expenditures can be greatly reduced.

1.3 Conclusions

The LD_{50} of GDNP in female rats in tap water solution is 720 milligrams per kilogram (mg/kg). Daily oral exposure to 500 milligrams per kilogram per day (mg/kg-d) of GDNP for 14-days causes weight loss, increased liver and spleen organ weights, and adverse histopathologic events in kidney and spleen. These adverse events were not observed in animals receiving lower doses of GDNP.

The LOAEL from oral exposure to GDNP for 14-days, as determined from this study, is 500 mg/kg-d based on significant adverse event of increased spleen and liver weight ratios and adverse histological alterations in the spleen and kidney. The NOAEL is therefore determined to be 152 mg/kg-d where no adverse events were found.

1.4 Recommendations

The acute and subacute oral toxicity of GDNP is low and is not limiting to the continued development of GDNP. The acute/subacute toxicity of of GDNP is two orders of magnitude lower than RDX.

2 References

See Appendix A for list of references.

3 Authority

Military Interdepartmental Purchase Request (MIPR) No. 0BDAT4D100. This study addresses, in part, the environmental safety and occupational health (ESOH) requirements outlined in Department of the Army (DA) Regulation 200-1 (DA 2007); DA Regulation 40-5 (DA 2007); and DA Regulation 70-1 (DA 2003); Department of Defense Instruction (DoDI) 4715.4 (DODi 1998); and Army Environmental Research and Technology Assessment (AERTA requirement PP-3-02-04 (AERTA. 2009). This study was performed as part of an on-going effort by the U.S. Army EQT, OEP to reduce or eliminate the environmental impact from life-cycle use of new chemical formulations proposed for use in weapon systems or platforms. This program is under the direction of the U.S. Army Research, Development and Engineering Command (USARDECOM) Environmental Acquisition Logistics & Sustainment Program (EALSP; Mr. Erik Hangeland) and EQT P2 Chair (U.S. Army Research Laboratory (USARL); Dr. John Beatty).

4 Background

RDX, the Army's long-standing primary explosive, is accumulating in the environment surrounding training ranges. RDX is a known environmental toxicant with a USEPA acute oral minimum risk level (MRL) of 60 micrograms per kilogram per day (µg/kg-d) based on its epileptiform seizure neurotoxicity in humans and rodents (Burdette et al. 1988; Kasuske et al. 2009; Stone et al. 1969; Williams et al. 2011). A reference dose (RfD) of 3 µg/kg-d has been established based on prostatic inflammation in rodents. RDX is also classified as a possible carcinogen (Lish et al. 1984; Parker et al. 2006).

Military training operations require the use of unique substances such as energetics, explosives, and propellants. Encroachment of local communities on military installations has increased the potential for human exposure, and recently-discovered health concerns with these substances can slow or halt training, resulting in affects on military readiness, and possibly costly environmental restoration actions. Historically, military munitions were developed based strictly on their effectiveness on the battlefield. Formal toxicity screening was not required, and the environmental impacts were discovered long after implementation, leading to costly remediation efforts. Over the past 30 years, the need to balance effectiveness with sound environmental stewardship has become a greater priority. Toxicity evaluations are now being performed during the RDT&E stage rather than following acquisition when design modifications are more costly. Determination of

toxicological and environmental fate parameters will help in the assessment of overall life-cycle costs and potential health and safety issues involved in the fielding of a new munition item..

The Army EQT OEP is dedicated to finding replacements for RDX that will reduce or eliminate the health risks from environmental exposure and will reduce adverse ESOH effects; RDX adversely affects the readiness and costs associated with training (USACHPPM 2007a). By identifying ESOH effects early in the acquisition process, unacceptable replacement compounds can be identified and unnecessary budget expenditures can be greatly reduced.

USARDECOM-sponsored work with Physical Sciences Inc. (Andover, MA) and BAE Ordnance Systems (Kingsport, TN) developed GDNP as a possible replacement for RDX. This document reports the procedures, findings, and conclusions of a progressive series of two oral toxicity studies performed with GDNP in laboratory female rats. The series consisted of a Sequential stage-Wise Probit, (SSWP) acute procedure and a 14-day repeated dose study. Such investigations identify effect levels and define target organs to determine how the mammalian toxicity of the material compares to currently fielded explosives.

The CHPPM Good Laboratory Practice Policy—Policy Memorandum 74 states—

> *All experiments and studies conducted by any element of the USAPHC Portfolio of Toxicology will be compliant with the applicable Good Laboratory Practice (GLP) guidelines reflected in the following regulations:*
>
> *(1) Title 21 Code of Federal Regulations (CFR) Part 58, Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.*
>
> *(2) Title 40 Code of Federal Regulations (CFR) Part 160, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Good Laboratory Practice Standards: Final Rule.*
>
> *(3) Title 40 Code of Federal Regulations (CFR) Part 792, Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards: Final Rule* (Memorandum 9 November 2009,).

According to this policy and the fact that the results of these assays may be used in regulatory decisions involving the USEPA, the studies were conducted in compliance with GLP standards and followed the appropriate regulatory testing guidelines depending on the test compound (USAPHC (Prov). 2009). A Quality Assurance Statement is provided in Appendix C, and an Archive Study Personnel Statement is provided in Appendix D. Table 1 identifies the critical events and dates of this study.

Table 1. Critical Dates of Acute and 14-Day Studies

Critical Event	Date of Event
Animal Use Protocol Approved	May 17, 2011
SSWP Animals Received	June 8, 2011
Study Start	June 13, 2011
Experimental Start	June 14, 2011
SSWP Necropsies	July 5, 2011
14-Day Animals Received	July 20, 2011
14-Day Study Start	July 22, 2011
14-Day Necropsies	August 11, 2011
Experimental Completion	August 11, 2011
Study Completion	

5 Materials

5.1 Test Substance

The molecular structure of GDNP is shown in Figure 1. GDNP is a light yellow medium to fine grain powder. The material was developed by Physical Sciences Inc. (Andover, MA 01810, SBIR Contract No. W911QX-06-C-0138), and supplied by BAE Ordinance Systems (Kingsport, TN); Batch # 1083-97. The compound was certified to be >99 percent pure as measured by differential scanning calorimetry (DSC) and 100 percent pure by nuclear magnetic resonance (NMR) spectral analysis. Analytical validation of GDNP purity was accomplished by high performance liquid chromatography (HPLC, CAD Method 98.2) by the Chromatographic Analysis Division (Explosives Team), Laboratory Sciences Portfolio (LSP) of the Army Institute of Public Health (AIPH).

Preliminary studies indicated that GDNP dissolves slowly in water with stirring up to 100 milligrams per milliliter (mg/mL). However, for stage 1 of the acute study (described below) dry crystalline GDNP was weighed out into plastic pans on a per animal basis. At the higher doses of 1 and 2 grams per kilogram (g/kg), the compound did not dissolve readily in water in the weigh pan. A suspension of the 1 g/kg dose could be pulled into the dosing syringe and administered to the rat. However, the 2 g/kg dose was too viscous and could not be administered. In stage 2 of the acute study, prolonged water exposure of the compound weighed per animal was attempted to improve solubility. However, upon standing in water, the crystal structure of the solid appeared to change, becoming a smooth, hard, insoluble mass. For stage 3, the GDNP was suspended in corn oil; suspension was difficult due to necessity of extensive grinding. For stage 4, a 100 mg/mL stock of GDNP was prepared in tap water and animals were dosed with this solution.

For stability tests, a solution of 25 mg/mL was prepared in tap water and stored at room temperature. Samples taken up to 18 days after preparation were analyzed by HPLC as described above; the results indicated no loss of compound under these conditions, i.e., GDNP is stable at room temperature up to 18 days. For the subacute study, half-log serial dilutions were prepared from a stock concentrate approximating 50 mg/mL. The concentration of the lowest dilution was validated by HPLC for GLP compliance and the concentrations of the higher dosing solutions determined by back calculation. On day 8 of the sub acute study, a rust-colored flocculent precipitate was observed in the highest concentration dosing solution. To make sure that the dosing concentration was not affected by the precipitate, a fresh stock was prepared and validated by LSP. The volume of solution administered to each rat was adjusted, based on animal weight, so that the rats continued to receive the intended dose.

Guanidinium 3,4- dinitropyrazolate

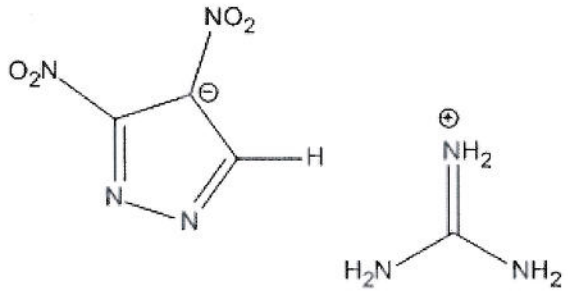

Figure 1. Molecular Structure of GDNP

5.2 Animals[1]

All studies were conducted using young adult female Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA). At the time of their arrival, the animals for the acute study were 8-weeks old, and the animals for the 14-day study were 6-weeks old. The Attending Veterinarian examined the animals and found them to be in acceptable health. The animals were quarantined for a minimum of 5 days after their arrival in this facility. All rats were maintained in a temperature-, relative humidity-, and light-controlled room. The conditions were 64-79 degrees Fahrenheit (°F), 30 percent to 70 percent relative humidity with a 12-hour light/dark cycle (USACHPPM. 2006). A certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and drinking quality tap water were available *ad libitum* (USACHPPM. 2006). Rats were housed individually in suspended polycarbonate boxes with Harlan Sani-Chip® bedding. Each rat was uniquely identified by number using cage cards only for the acute study and both cage cards and tail marking for the 14-day study. (Teklad® is a registered trademark of Harlan, Teklad; Harlan® Sani-Chip is a registered trademark with P.J. Murphy Forest Products Corporation.)

5.3 Quality Assurance

The USAPHC (Prov) Quality Systems Office audited critical phases of this study. Appendix B provides the dates of these audits along with the audited phase and date reported to Management and the Study Director.

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[1] Animal use procedures were approved by the Animal Care and Use Committee at the United States Army Public Health Command (USAPHC). Animal care and use was conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, D.C. 1996), and in accordance with all applicable Federal and DOD regulations. The USAPHC animal care program is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

5.4 Study Personnel

Appendix C contains the names of persons contributing to the performance of these studies.

6 METHODS

6.1 Acute Study (Sequential Stage-Wise Probit, SSWP)

The objective of this phase of the study was to determine the median acute oral lethal dose (LD_{50}) and slope of GDNP in the female Sprague-Dawley rat. Results are useful for relative toxicity comparisons and to determine dosage levels for the subacute (14-day) study. The general procedures of this acute study followed the USEPA Health Effects Test Guidelines for Acute Oral Toxicity (OPPTS 870.1100) (USEPA 1998).

The SSWP procedure was used to determine the estimated oral LD_{50} and confidence interval of GDNP to the Sprague-Dawley rat (Feder, Hobson, et al. 1991; Feder, Olson, et al. 1991). The procedure was completed using four separate stages of dosing.

All animals were fasted overnight prior to dosing and for up to 4 hours post-dosing. Doses for the first stage of the acute tests were 125, 250, 500, 1000, and 2000 mg/kg. All doses were calculated based on body weights taken immediately prior to dosing. The amount of GDNP appropriate for each rat was weighed individually in a weigh pan. Animal room filtered tap water was added up to the maximum allowed (10 mL/kg); the GDNP in solution/suspension was pulled into the gavage syringe, and administered by oral gavage using a 16 gauge x 2-inch stainless steel gavage needle; maximum volume did not exceed 10 milliliters per kilogram (mL/kg) (EPA 1998).

In the second stage, there were difficulties encountered due to apparent insolubility of GDNP at higher intended doses of 500 to 1000 mg/kg (see Section 5.1). A stage 3 attempted suspensions in corn oil as the vehicle. Gavage dosing with this vehicle was also challenging. The final stage 4 dosing used filtered tap water solutions of GDNP. A solution of 100 mg/mL was prepared; the concentration was verified by HPLC. In this final stage, 3 rats each were given a single dose of 660, 760, or 870 mg/kg.

Following administration of the test compound for each phase of the acute test the rats were observed for 14 days. All clinical signs or incidences of death were recorded on a daily basis. Individual body weights were recorded daily (5 days a week) throughout the 14-day observation period to determine recovery.

Surviving animals were euthanized on day 14 and submitted for gross pathological examination. The estimated LD_{50} value was then calculated using the number of surviving animals at the end of the 14-day observation period for each stage of dosing.

6.2 Subacute 14-Day Oral Repeated Dose Toxicity Study

Upon evaluating the results of the SSWP procedure, a 14-day repeated dose oral toxicity study was conducted in female rats according to the Toxicology Portfolio SOP for 14-day Oral Toxicity Study in Rats (USACHPPM. 2008)

Seventy female Sprague-Dawley rats, obtained at 6 weeks of age, were used for this phase of the study. Following a 5-day quarantine/acclimatization period, the animals were randomly distributed using an EXCEL™ randomization program into 7 treatment groups consisting of 10 female rats

each. Dosage levels were set at 0, 1.3, 4.2, 13.9, 45.9, 151.5 and 500 mg/kg-d. The animals were then divided into three evenly distributed experimental groups; the start dates for each group were staggered over a period of three days to facilitate scheduling of necropsies.

Using filtered tap water from the animal room as the vehicle, the six dosing solutions were prepared by making 1:3.3 (approximately half-log) serial dilutions beginning from a concentrated stock of 50 mg/mL. The final dilution, 0.13 mg/mL was verified by HPLC. The doses were administered daily, 7 days per week for a total of 14 doses. A 16 gauge x 2-inch stainless steel gavage needle was used to facilitate oral dosing. The solution/suspensions were sampled and analyzed by HPLC to verify concentrations and stability prior to the first day of dosing.

On day 8 of the subacute study, a rust-colored flocculent precipitate was observed in the highest dosing solution. To make sure that the dosing concentration was not affected by the precipitate, a fresh stock was prepared and validated by HPLC. The volume of solution administered to each rat was adjusted, based on animal weight, so that the rats continued to receive the intended dose.

Body weights were recorded on days -1, 0, 1, 3, 7, and 14. Food consumption based on change in feeder weights was monitored weekly. Animals were observed daily for toxic signs and morbidity. Water consumption was not monitored during this study. All data were recorded onto hardcopy spreadsheets and transcribed to an EXCEL™ spreadsheet for computer analysis.

At the end of the 14-day study period, the rats were anesthetized with carbon dioxide gas, and blood was collected by intracardiac puncture. The animals were then euthanized using carbon dioxide. Clinical chemistry and hematology values were determined from all useable samples. The brain, heart, kidneys, liver, ovaries, spleen, and uterus were removed and weighed for absolute organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios. Gross necropsies were completed on all terminal animals. The following parameters, by test group, were analyzed and compared to the controls: (a) body weights; (b) weight gains; (c) food consumption; (d) absolute organ weights; (e) organ-to-body weight ratios; and (f) organ-to-brain weight ratios.

Liver, spleen, kidney, ovary, uterus, and tissues noted with gross abnormalities were collected and appropriately preserved in 10 percent buffered formalin, selectively trimmed and placed in cassettes labeled with protocol number, animal identification number, and laboratory assigned accession number. Cassettes were placed in labeled formalin filled bottles and transported to the US Army Medical Research Institute of Chemical Defense (USAMRICD) for processing. Tissues were routinely processed and paraffin embedded. All processed and embedded tissues were microtomed at 5µm thick, automatically stained with hematoxylin and eosin, and coverslipped. The pathologist examined slides for compound-induced histopathologic changes via light microscopy. Prevalence and severity of findings were graded in comparison to controls. Findings are classified as none, minimal, mild, moderate or severe.

Hematology (Cell-Dyn 3700 Hematology Analyzer, Abbott Laboratories, Abbott Park, Illinois 60064) variables included: white blood cell count (WBC), WBC differential (percent neutrophils (NEU %N), percent lymphocytes (LYM %L), percent monocytes (MONO %M), percent eosinophils (EOS %E), percent basophils (BASO %B), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV).

Clinical Chemistry (VetTest 8008 Chemistry Analyzer and VetLyte Na, K, Cl Analyzer, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092) variables included: albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (non-fasting) (GLU), globulin (GLOB), lactate

dehydrogenase (LDH), phosphorus (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chlorine (Cl).

Statistical analysis was done using Prism Version 4.03 (GraphPad Software, Inc.). Experimental results were presumed to have a normal distribution. For variables that were measured only at the end of the study, the dosage groups were compared using a one-factor analysis of variance (ANOVA) assuming a normal distribution and equivalent variance between treatments. Organ-to-brain and organ-to-body-weight ratios were calculated and analyzed similarly to the other parameters measured at the end of the study with the ratio transposed by factors of 10 to transform the ratios to numbers greater than 1, i.e., normalization. If the ANOVA was significant, Dunnett's multiple comparison test was used to determine statistical significance ($p \leq 0.05$) of individual GDNP dosage groups to the control group.

7 RESULTS

7.1 Analytical Chemistry

The analytical chemistry results from both the acute and 14-day studies are contained in Appendix D. GDNP was stable at room temperature for the 18-day period of the study. The results of the dosing solution concentration samples were consistent with the calculated nominal concentrations. As the validated concentrations of the second batch of high dosage solutions (see Methods) indicated a more concentrated stock solution, adjustments were made to the dosing volumes for the final 5 days of dosing.

7.2 The Sequential Stage-Wise Probit Procedure

The results of the SSWP procedure are presented in Appendix E. Dosing was confounded by the solubility characteristics of GDNP (see Section 5.1 Test Substance). The preliminary stages indicated an LD_{50} above 500 mg/kg. The affected animals quickly (within 20 minutes) became sluggish and progressively sedated in a dose-dependent manner. One animal showed an indication of diarrhea. Most animals became inactive on the cage floor, exhibited 2 to 3 brief convulsions and died, becoming immediately tetanic, i.e., clear stiffening of core body and extremity musculature. This immediate tetany was not as severe as the slower rigor mortis because the tetany was not sufficient to enable planking when the animal was held by the tail as is characteristic of rigor mortis. Calculations based on the results of the stage 4 dosings indicated the LD_{50} of GDNP in tap water solution to be 718 mg/kg, rounded to 720 mg/kg. The slope of the dose-response curve is very steep, but confidence intervals were not able to be derived due to the small sample size of the final dosing groups using GDNP tap water solution.

7.3 14-Day Oral Repeated Dose Toxicity Study

A summary of results and raw data for the 14-day oral repeated dose toxicity study are presented in Appendices F-L.

On the first days of dosing, lethargy was observed in the first 30 min after dosing in about half of the animals; sedation and death were not observed. Urine from dosed animals was intensely yellow in the 500 mg/kg-d group the color intensity decreased with decreased dose, and was not noticeably different from the vehicle control group below 13.9 mg/kg-d. After about four days of dosing, the effect of high dosage GDNP on activity, compound-induced lethargy, was less apparent and was not observed in the second week of dosing. Lethargy was not observed at anytime at dosages below 500 mg/kg-d. No other clinical signs were observed in any of the dosing groups.

The net body weight change of the animals increased similarly with time for all dosage groups except for the 500 mg/kg-d dosage group. Weight gain was reduced in this group for the first week of dosing (see Appendices G and H). Over the second week of dosing, the 500 mg/kg-d group gained weight faster than the other groups including the control group (Appendix H) so that by 13 days, the average weight of the 500 mg/kg-d group was not different from control (Appendix G). The effect of high dosage GDNP on body weight was reflected in food consumption rates (Appendix I). The amount of food eaten by the 500 mg/kg-d group during the first week was less than the control group and only in animals from this dose group. The amount of food eaten during the second week was similar to the other groups, including the control group. However, the total food consumption over the 2 weeks of dosing was still less from rats in the 500 mg/kg-d dose group (Appendix I).

Gross lesions noted at necropsy included dark red spleen, enlarged spleen or liver, pale liver or kidneys, dilated uterus (hydrometra), enlarged mesenteric lymph nodes and prominent Peyer's patches of the small intestine. A few of the grossly enlarged mesenteric lymph nodes and Peyer's patches correlated histologically with lymphocytic hyperplasia. All gross observations of hydrometra correlated with histologic uterine dilatation. The amount of fluid within the uterine lumen varies throughout the estrus cycle. During proestrus the uterus normally becomes distended with watery fluid, appearing grossly as a "hydrometra" (Leininger and Jokinen 1990). Hydrometra was not considered an adverse finding. There were no histologic correlates with grossly observed enlarged pale spleens and livers.

Differences were observed between the dose groups and the control group in mean organ weights, and organ to body and brain weight ratios for the liver, spleen, uterus and ovaries (Appendix L). The differences were similar in all three methods of analysis. There was a distinct increase in liver (38-45 percent) and spleen (23 to 47percent) weights in rats from the 500 mg/kg-d dose group. There was a consistent decrease (30 to 60 percent) in uterine weight in females from rats in the lowest dose group; there was a trend for a dose-related decrease in uterine weight. The impact of dose on ovary weight was inconsistent; there was an increase (16 to 20 percent) in ovary weight compared to controls at the intermediate dosages of 13.9 to 151.5 mg/kg-d, with no impact of the 500 mg/kg-d dosage, i.e., ovary with in this high dose group was no different than controls (Appendix L).

Histopathology revealed several abnormalities (Figure 2, Appendix M). Based on incidence and severity, lesions associated with repeated dose exposure to GDNP were noted in a dose dependent manner in the kidney and spleen. Extramedullary hematopoiesis (EMH), increased presence of hematological precursors in the splenic red pulp, was noted to be increased, compared to vehicle controls, in the 500 mg/kg-d group (6/10 moderate, 3/10 mild and 1/10 normal) (Figure 2A). In the kidney, basophilic tubules, indicating tubular regeneration, with or without tubular degeneration and luminal debris was noted in the 500 mg/kg-d group, as compared to vehicle controls (3/10 minimal, 5/10 mild and 2/10 moderate) (Figure 2B).

EMH is present in the normal spleen of rats and is more common in young versus aged animals (Suttie 2006). Findings of minimal to mild EMH can be within the degree of normal variation. However, a dose effect was noted in severity and prevalence in the 500 mg/kg-d group. Increases in EMH compared to vehicle control spleens may be the result of a hematoxic insult.

Basophilic tubules noted were often small, had increased epithelial cells, a distinct blue cytoplasm, occasional mitotic figures and large "open" nuclei. Occasionally, there was tubular epithelial degeneration and luminal eosinophilic debris, indicating recent degeneration and necrosis of

epithelial cells often along the proximal tubule. Luminal debris was only noted within the 500 mg/kg-d group. This may suggest repeated injury of different tubules from treatment with regeneration as

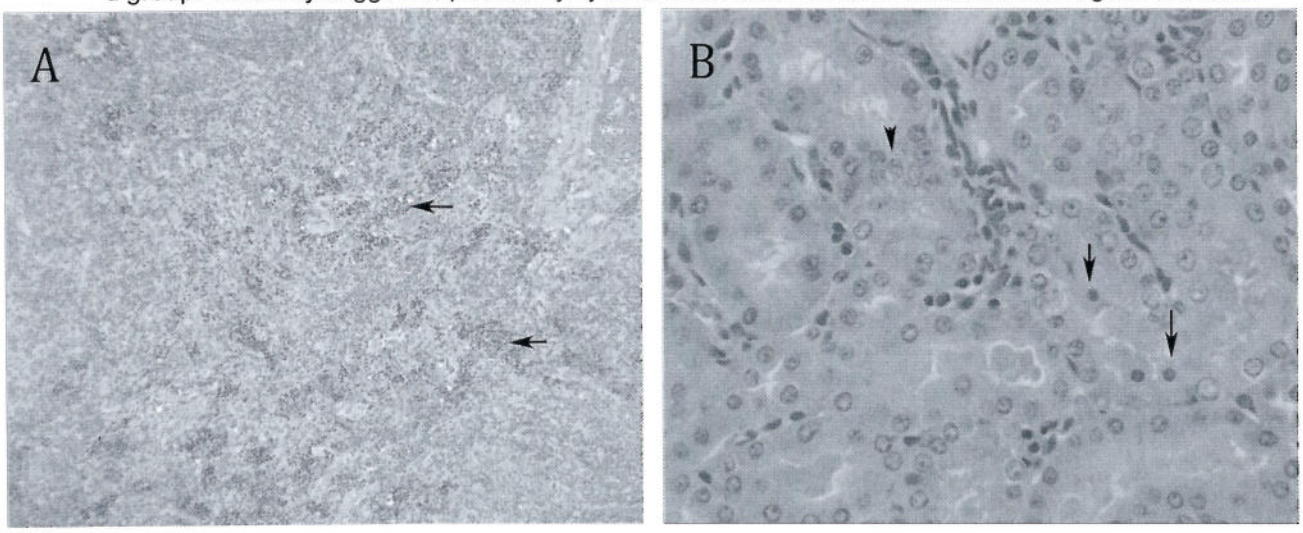

Figure 2 – Subacute Histopathology. Extramedullary hematopoeisis was increased in the 500 mg/kg-d group (Figure 2A, 10x). Throughout the red pulp, there are numerous erythropoetic precursor cells, evidenced by round dark nuclei with indiscernible cytoplasm (arrows). In the kidney, necrotic tubular cells, hypereosinophilic cells with dark, shrunken nuclei, are noted within tubular lumen (long arrows). Basophilic, regenerative cells are identified by large, "open" nuclei with epithelium often stacked upon each other (arrowhead) (Figure 2B, 40x).

opposed to isolated tubular injury or turnover. Additionally, the severity was increased in this group. This finding is most likely associated with repeated damage to tubules over the 14 day period and is likely treatment-related.

Hepatocellular centrilobular hypertrophy is an enlargement of hepatocellular cytoplasm in response to enzyme induction and is considered an adaptive response to chemical stress (Thoolen et al. 2010). In the 500 mg/kg-d group, 7 out of 10 rats demonstrated a minimal-to-mild response. This lesion was treatment related but was considered adaptive and not adverse at this time. Excessive hypertrophy can result in hepatocellular degeneration and necrosis.

Other findings that occurred infrequently or comparable to controls were considered to be background lesions or of minimal significance and not treatment-related. These lesions were found in the spleen, mesenteric lymph nodes and Peyer's patches as lymphoid hyperplasia. Extramedullary hematopoiesis of the liver, lymphohistiocytic hepatic infiltrates, renal mineralization, and uterine dilatation were also found and not attributed to treatment.

There were few lesions of note that occurred within individual animals. Grossly, two rats (11-0893 and 11-0916) had abdominal adhesions involving the serosal surface of the stomach and/or diaphragm, liver, and spleen. Microscopically, severe necrotizing and pyogranulomatous, transmural, gastritis was noted with granulation tissue. These lesions were most likely due to a repetitive dosing injury causing full thickness gastric damage and subsequent healing with granulation tissue and its adhesion to surrounding serosal surfaces of organs.

Analysis of the clinical chemistry results revealed differences for TP, ALB, ALT, BUN, CREA, and CHOL compared to the vehicle control group (Appendix J). The TP and ALB concentrations were increased 15 percent and 20 percent, respectively in dose groups of 45.9 mg/kg-d and above. ALT was significantly increased 70 percent and CREA was increased 83 percent in the 500 mg/kg-d dose group. CHO was increased significantly, albeit only 6 percent in the 45.9 mg/kg-d and above. BUN level exhibited significant 22 percent decreases in the 45.9 and 151.5 mg/kg-d dosages, but was elevated 7 percent above control levels in the 500 mg/kg-d dose group (Appendix J).

Statistical analysis of the hematology results revealed a dose-related increase in the concentrations of WBC (K/µL) reaching a 1.9-fold increase in the 500 mg/kg-d dose group (Appendix K). Similarly, there were significant increases in the absolute concentrations of NEU, BASO, EOS and LYM when compared with data of rats from the control group. The increases were predominantly neutrophils and basophils (4-5 fold increase) with corresponding increases in the percent contribution to the total WBC population. Interestingly, although there was a 1.5-fold increase in the absolute concentration of lymphocytes, the contribution to the total WBC pollution was decreased 15 percent. There was no change in the absolute density of RBCs, but a slight decrease (6%) in HCT and HGB with a 13% increase in RDW (Appendix K).

8. Discussion

The LD_{50} of guanidinium free base, the neutral form of the guanidinium cation of GDNP, is 475 mg/kg in female rats (HSDB 2010). The hydrochloride and nitrate salts of guanidinium have LD_{50}'s in female rats of 774 mg/kg (Morgan et al. 1985) and 729 mg/kg (Mullen et al. 1989), respectively. Clinical signs were observed in both the gastrointestinal (GI) tract and the central nervous system-neuromuscular junction (CNS-NM). Symptoms referable to the GI tract included increased salivation, hunched posture, and diarrhea. CNS-NM signs included disorientation, increased startle reflex, hyperactivity, jumping behavior, presence of tremors/twitching, and depression of grasping and righting reflexes. Interestingly, nitroguanidine is not particularly toxic after oral administration either acutely (LD_{50} is > 2 g/kg in rats) (IRIS 2011; Lewis 2004) or subchronically at doses as high as 1000 mg/kg-d (Morgan et al. 1988). TOPKAT modeling software estimates the LD_{50} of 3, 4-dinitropyrazolate, the anion of GDNP, to be 162 mg/kg.

In the present study, the LD_{50} of GDNP in tap water solution in female rats is 720 mg/kg; a confidence interval was not able to be determined. Progressive disorientation and lethargy were consistently observed at doses approaching this LD_{50}. This is similar to the LD_{50} reported for guanidine nitrate (Mullen et al. 1989). Most of the mass of guanidine nitrate is carried by the guanidine cation whereas guanidine carries about half of the mass of GDNP. This suggests that the 2, 4 dinitropyrozolate anion of GDNP contributes to the acute toxicity. The slope of the LD_{50} curve appears to be fairly steep and dependent on the vehicle used; 500 mg/kg dose of GDNP in corn oil was lethal while the same dose in tap water solution was not. In tap water solution, a dose of 1000 mg/kg was 100 percent lethal. The contribution of vehicle to GDNP lethality is not clear.

In the subacute study, the highest dosage used was 500 mg/kg-d. This dose was just below the LD_{50}, and was hypothesized to have a cumulative affect over 14 days that would result in mortality. Animals in this dose group exhibited moderate lethargy during the first 30 min after dosing, but none of them lost consciousness. Over the course of the 14 days of dosing, the lethargic response to dosing became less apparent. Similarly, the effect of high dose GDNP impacted food intake and body weight gain during the first week of dosing. However, this effect was observed during the second week of dosing where the animals recovered from the weight loss and gained weight faster than the other dose groups. These observations suggest that rather than GDNP having a

cumulative toxic effect, the animals appear to become tolerant to GDNP and less responsive to adverse effects of the compound.

The urine of animals dosed with GDNP was more intensely yellow than vehicle-treated rats. This was apparent in the dose groups of 13.9 mg/kg-d and above. The intensity of the yellow urine increased in a dose-responsive manner to a very intense yellow in the 500 mg/kg-d group. The presumptive GDNP-derived excrement was present after 24 hours of the last dose indicating that urinary clearance of GDNP is longer than 24 hrs. Presumably, plasma levels of GDNP never accumulated to lethal levels as none of the rats died during the course of the 14 day study.

Despite evidence of compound tolerance in clinical signs, food intake, and weight gain, consistent effects were observed on white blood cell concentrations, blood chemistries and organ weights. WBC counts were increased several fold above control. Lymphocyte concentration was an exception, and was decreased in percent contribution to the total WBC population. Both liver weight and plasma ALT were increased 40 and 70 percent, respectively, in the 500 mg/kg-d dosage group. The biologic significance of the increased CREA (83%) in the 500 mg/kg-d group and the decreased BUN (22%) in the 152 mg/kg-d group is not clear, particularly when there was no apparent change in kidney weight;.

The neutrophil counts of two animals within the 500 mg/kg-d group (11-0893 and 11-0916) were elevated compared to all vehicle controls and other treatment groups. These two animals demonstrated severe pyogranulomatous gastritis accounting for their elevated neutrophil counts. These elevated counts elevated the neutrophil mean for their group. Hematologic values in the 500 mg/kg-d group correlate with a mild anemia as compared to coincident and historical controls. Decreased hemoglobin, red blood cell count and hematocrit are indicative of a change in the circulating red blood cell mass. Additionally, red cell distribution width (RDW) was highest in this group; RDW is indicative of a variation of red cell size and will be elevated in anemias with significant cell size differences.

An increase in serum ALT activity in the range of 2-4X or higher in individual or group mean data when compared with concurrent controls should raise concern as an indicator of potential hepatic injury unless a clear alternative explanation is found (Boone et al. 2005). A mild elevation of ALT was noted in the 500mg/kg group but there was no concurrent microscopic evidence. The mild increase may be due to concurrent hepatic microsomal induction (Boone et al. 2005).

Rats in this study exhibited some microscopic and hematologic changes similar to rats orally exposed to other explosives, such as 2,4,6-Trinitrotoluene (TNT). With TNT, dose dependent anemia was seen in treated rats with reductions in hemoglobin, hematocrit and red blood cell counts (Yinon 1990). The 500 mg/kg-d group demonstrated reductions in all three blood values. Additionally, EMH of the spleen was most evident in this group; EMH can occur under conditions of demand such as anemia.

Nephropathy (chronic progressive nephropathy – CPN) is a spontaneous disease of the F344 strain of rat, with minimal lesions of scattered foci of tubular regeneration, occurring as early as 5 months of age (Montgomery and Seely 1990). Exacerbation of lesions of CPN represents the most commonly-reported minimal expression of nephrotoxicity of the rat. Most nephrotoxicants, at doses below those that induce overt evidence of nephron or tubular injury, cause or exacerbate injury morphologically the same as CPN (Haschek et al. 2010). Tubular basophilia, i.e. tubular regeneration, was evident minimally in all dosage groups. The 500 mg/kg-d group noted tubular regeneration in the minimal, mild and moderate ranges with 5/10 rats demonstrating tubular debris and/or tubular epithelial degeneration. The renal lesions in the 500 mg/kg-d appear to be treatment

related and may be an exacerbation of underlying early CPN, due to the minimal presence of tubular basophilia in all groups, including the vehicle control.

9. Conclusions

The LD_{50} of GDNP in female rats using tap water as a vehicle is 720 mg/kg. Daily oral exposure to 500 mg/kg-d for 14-days of GDNP causes weight loss, increased liver and spleen organ weights, and adverse histopathologic events in kidney and spleen including increased EMH in the splenic red pulp, and basophilic tubules in the kidneys. These adverse events were not observed in animals receiving lower doses of GDNP.

The LOAEL from oral exposure to GDNP for 14-days, as determined from this study, is 500 mg/kg-d based on significant adverse event of increased spleen and liver weight ratios and adverse histological alterations in the spleen and kidney. The no-observed-adverse-effect-level is therefore determined to be 152 mg/kg-d where no adverse events were found.

10 Recommendations

The acute and subacute oral toxicity of GDNP is low and is not limiting to the continued development of GDNP.

11 Point of Contact

Dr. Larry Williams, the principal investigator, is the point of contact for this project. He may be reached commercial 410-436-3980.

Appendix A

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Appendix B

Authority

Military Interdepartmental Purchase Request (MIPR) No. 0BDAT4D100.

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HEADQUARTERS, U.S. ARMY MEDICAL COMMAND
Fort Sam Houston, TX 78234-6007
261600R February 2010

APPENDIX C

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. 87-XE-0E9V-30-11-05-01, Effects of Oral Guanidinium 3,4-dinitropyrazole (GDNP) Exposure to Female Rats *(Rattus norvegicus)*, February 2012, the following critical phases were audited by the Quality Systems Office:

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol GLP Review	02/16/2011	02/16/2011

IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
LD50 - 1st Stage - Pre-Procedural Provisions	06/14/2011	06/21/2011
LD50-1st Stage-Post Dosing Test System Observation	06/14/2011	06/21/2011
LD50-1st Stage-Test Article Control and Dosages	06/14/2011	06/23/2011
LD50 - 2nd Stage - Test System Identification	06/16/2011	06/23/2011
LD50 - 2nd Stage - Oral Gavage Technique	06/16/2011	06/23/2011
LD50 - 2nd Stage Dose Selection	06/16/2011	06/23/2011
LD50 - Final Stage - Test Article Administration	06/20/2011	07/01/2011
LD50 - Dosing Solution Concentration Verification	06/20/2011	07/01/2011
LD50 - Compliance with Study Protocol	06/20/2011	07/01/2011
LD50 - 14 day Observation Procedures	06/29/2011	07/01/2011
LD 50 determination - Study Endpoint criteria	07/06/2011	07/08/2011
14-Day Pre-Study Test Article Mixing and Control	07/22/2011	08/02/2011
14-Day Test System Identification and Food Supply	07/22/2011	08/02/2011
14-Day Dosing Solution Concentration Verification	07/22/2011	08/02/2011
Compliance with Study Protocol Modification	07/22/2011	08/02/2011
14 Day - Randomization Procedures	08/02/2011	08/09/2011

APPENDIX C

For: Toxicology Study No. 87-XE-0E9V-30-11-05-01, Effects of Oral Guanidinium 3,4-dinitropyrazole (GDNP) Exposure to Female Rats *(Rattus norvegicus)*, February 2012, the following critical phases were audited by the Quality Systems Office:

IN-LIFE PHASE OF THE STUDY (Cont.)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Personnel Training Records	08/04/2011	08/09/2011
14-Day - Test Article Labeling and Administration	08/05/2011	08/15/2011
14 day - Test System Environmental Conditions	08/05/2011	08/15/2011
14 Day - Pre-Necropsy Procedures	08/10/2011	08/11/2011
14-day Euthanasia and Necropsy Procedures	08/11/2011	08/15/2011
14 Day Study Final Endpoint Criteria	08/11/2011	08/15/2011
MRICD Tissue Processing - Facility/Process Audit	10/20/2011	10/20/2011

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Final Study Report Review	02/03/2012	02/08/2012
Study Raw Data Review	02/03/2012	02/08/2012

Note 1 All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2 In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

Note 3 This report has been audited by the Quality Assurance Unit (QSO), and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver
Quality Assurance Specialist, QSO

02/08/2012
Date

Gene Sinar
Team Leader, QSO

2/8/2012
Date

Appendix D

Archives and Study Personnel

D-1. Archives

All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in the storage facilities of the Toxicology Portfolio, AIPH, for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

Records on the test system will be archived by the Toxicology Portfolio, for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

The present study used the laboratory project number: 87-XE-0E9V-11 for all filings.

The protocol, raw data, summary data, and the final report pertaining to this study will be physically maintained within Building E-2100, USAPHC. These data may be scanned to a computer disk. Scanned study files will be stored electronically in Room 3010, Building E-2100, USAPHC, Aberdeen Proving Ground (APG), MD, 21010.

Archived SOPs and maintenance and calibration logbooks may be found in Room 1026, Building E-2100, USAPHC (Prov), APG, MD, 21010.

Archivist: Martha Thompson

D-2. Personnel

Management: COL Chris E. Hanson, Portfolio Director, Toxicology; Mark Johnson, Ph.D., Program Manager, Health Effects Research Program (HERP)

Study Director: Larry Williams, Biologist, HERP.

Quality Assurance: Michael P. Kefauver, Chemist, Quality Systems Office.

Appendix E

Analytical Data
Summary of 14-Day Analytical Results
Reported Concentrations (mg/mL)

Protocol No. 0E9V-30-11-05-01
Oral Toxicity of GDNP in Female Rats

Acute study

For Stage 3 of the acute study, a solution was prepared on June 17, 2011 with an intended concentration of 60 mg/mL and for Stage 4 on June 18, 2011with an intended concentration of 100 mg/mL. DLS analytically verified concentrations to be 57 and 94 mg/mL, respectively. LD_{50} was derived using the verified concentrations.

Sub Acute 1

On July 22, 2011 half -log serial dilutions were prepared from a starting stock with an intended concentration of 60 mg/mL. The final dilution was sampled for verification by DLS. The concentration was 0.13 mg/mL and used to back calculate the concentrations of all dosing solutions.

Sub Acute 2

On August 1, 2011, a second series of dilutions was prepared from a 60 mg/mL stock to refresh the top three dosing solutions. The lowest solution was sampled for analysis by DLS. The concentration was verified as 5.3 mg/mL and the concentrations of the dosing concentrations were back calculated.

Note: The DLS analytical contributing scientist reports for this study can be found with this study's raw data.

Appendix F

SEQUENTIAL STAGE-WISE PROBIT (SSWP): ORAL, RAT

Table F-1
Protocol No. 0E9V-30-11-05-01
Acute Oral Toxicity of GDNP in Female Rats

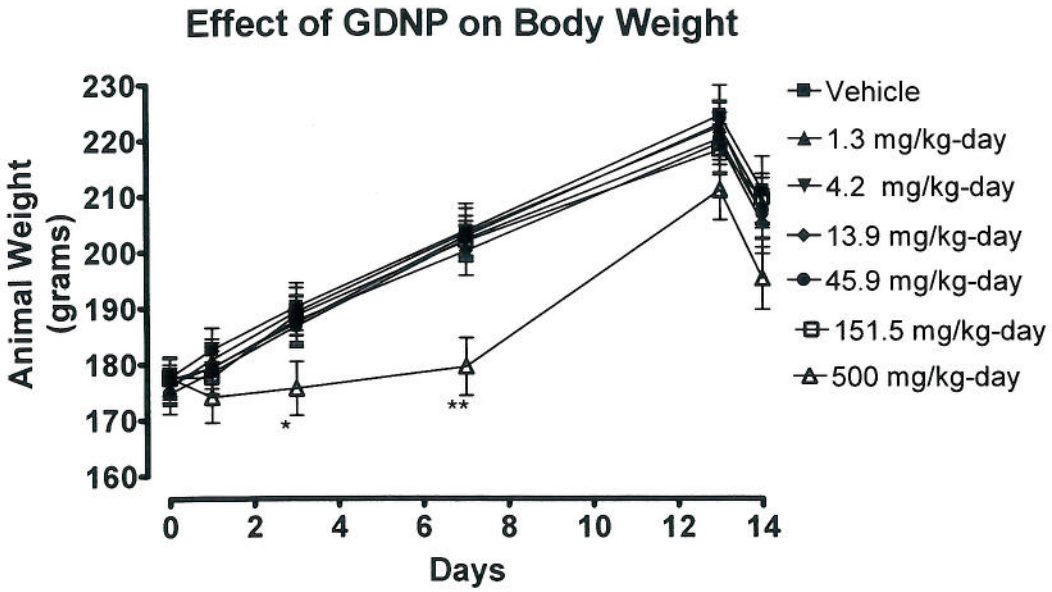
Study No. 87-XE-0E9V-11, Protocol No. 0E9V-30-11-05-01, SOP No. 17.08
Chemical Substance: Guanidinium 3,4-dinitropyrazole (GDNP)
Route: Oral Species: Sprague-Dawley Rat Sex: Female
Diluent: Tap Water or Corn Oil

Animal No.	Diluent	Dosing stage	Weight Kg	Nominal Dose mg/kg	Volume mL	Exposure Day Signs	Exposure Day Morbidity/Mortality
11-599	Water	1	0.173	125	1.5	N	N
11-600	Water	1	0.181	250	1.5	N	N
11-601	Water	1	0.171	500	1.5	Y	N
11-602	Water	1	0.177	1000	1.5	Y	Y
11-608	Water	2	0.193	630	1.5	Y	Y
11-609	Water	2	0.181	630	1.5	Y	Y
11-610	Water	2	0.190	630	1.5	Y	Y
11-611	Water	2	0.186	500	1.5	N	N
11-612	Water	2	0.189	500	1.5	N	N
11-603	Corn Oil	3	0.190	500	1.5	Y	Y
11-604	Corn Oil	3	0.184	500	1.5	Y	Y
11-605	Corn Oil	3	0.177	440	1.5	N	N
11-606	Corn Oil	3	0.183	440	1.5	Y	N
11-607	Corn Oil	3	0.184	440	1.5	Y	N
11-613	Water	4	0.196	390	1.18	N	N
11-614	Water	4	0.191	390	1.14	N	N
11-615	Water	4	0.191	475	1.39	Y	N
11-616	Water	4	0.194	475	1.44	Y	N
11-617	Water	4	0.194	580	1.78	Y	N
11-618	Water	4	0.195	580	1.76	Y	N
11-619	Water	4	0.194	1000	1.94	Y	Y
11-620	Water	4	0.177	660	1.17	N	N
11-621	Water	4	0.211	660	1.39	N	N
11-622	Water	4	0.208	660	1.37	N	N
11-623	Water	4	0.222	760	1.69	Y	N
11-624	Water	4	0.188	760	1.43	Y	N
11-625	Water	4	0.178	760	1.35	Y	Y
11-626	Water	4	0.189	870	1.64	Y	Y
11-627	Water	4	0.206	870	1.79	Y	Y
11-628	Water	4	0.202	870	1.76	Y	Y
Final stage 4 dosing solution verified at 94 mg/kg lowering theoretical LD_{50} of 767 mg/kg							
Study Conclusions – GDNP has an LD50 of 718 mg/kg							

APPENDIX G

SUMMARY OF 14-DAY BODY WEIGHTS AND INDIVIDUAL DATA

Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats



Note: Rats were fasted overnight prior to necropsy on Day 14. *$p \leq 0.05$

Figure G-1. 14-Day Body Weights and Individual Data

Table G-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Body Weights (grams)

		GDNP (mg/kg-day)						
Period		**Vehicle Control**	**1.3**	**4.2**	**13.9**	**45.9**	**151.5**	**500**
Day 0	**Mean**	177.7	176.0	176.5	177.3	174.8	177.7	177.6
	S.D.	10.8	10.7	10.9	12.9	11.6	10.5	12.0
	N	10	10	10	10	10	10	10
Day 1	**Mean**	182.6	180.8	179.4	179.3	178.5	177.6	174.1
	S.D.	12.1	11.8	11.8	14.4	12.3	9.2	14.3
	N	10	10	10	10	10	10	10
Day 3	**Mean**	190.2	189.3	187.3	187.7	186.7	188.7	***175.6**
	S.D.	13.5	13.7	10.8	13.5	11.9	11.3	15.1
	N	10	10	10	10	10	10	10
Day 7	**Mean**	203.8	203.4	202.9	200.2	202.3	202	****179.5**
	S.D.	15.4	14.0	11.3	14.0	13.1	11.1	16.2
	N	10	10	10	10	10	10	10
Day 13	**Mean**	224.5	222.5	222.9	219.6	220.3	218.3	211.1
	S.D.	16.9	13.3	13.4	16.9	14.7	14.2	17.039
	N	10	10	10	10	10	10	10
Day 14	**Mean**	211.0	208.8	206.4	205.2	206.9	209.6	195.3
	S.D.	19.6	14.2	13.1	17.2	13.6	13.9	17.6
	N	10	10	10	10	10	10	10

* $\rho \leq 0.05$ ** $\rho \leq 0.01$

Table G-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

14-Day Individual Body Weights (grams)

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
Vehicle Control	849	159	159	165	176	196	176
	853	165	170	176	187	207	187
	859	179	183	191	203	224	212
	860	196	203	215	230	253	240
	863	179	186	195	212	239	221
	874	187	192	199	221	242	231
	892	174	177	185	201	214	199
	899	186	188	192	204	223	211
	901	180	188	197	205	225	209
	905	172	180	187	199	222	224
Mean		177.7	182.6	190.2	203.8	224.5	211.0
SD		10.8	12.1	13.5	15.4	16.9	19.6

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
1.3 mg/kg-d	852	170	176	182	196	222	207
	857	170	170	178	192	205	189
	867	161	167	172	190	217	197
	870	181	183	200	210	214	204
	883	159	162	170	179	203	189
	894	181	190	200	217	232	221
	896	177	182	192	209	225	215
	897	191	198	212	226	244	229
	900	184	187	189	207	238	225
	907	186	193	198	208	225	212
Mean		176.0	180.8	189.3	203.4	222.5	208.8
SD		10.7	11.8	13.7	14.0	13.3	14.2

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
4.2 mg/kg-d	855	179	183	185	202	209	192
	866	175	180	194	216	236	219
	868	165	166	175	188	205	186
	873	166	167	171	193	203	199
	880	160	162	174	186	230	194
	895	173	175	186	205	219	210
	904	178	182	195	203	222	206
	906	191	195	200	212	235	222
	911	191	189	194	204	231	214
	913	187	195	199	220	239	222
Mean		176.5	179.4	187.3	202.9	222.9	206.4
SD		10.9	11.8	10.8	11.3	13.4	13.1

Table G-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

14-Day Individual Body Weights (grams)

Table G-2 (continued)

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
13.9 mg/kg-d	850	176	180	184	205	228	207
	862	152	152	162	170	183	169
	864	165	164	175	187	206	190
	865	170	175	181	204	225	205
	872	176	172	184	190	209	198
	881	180	185	194	207	220	204
	882	187	191	205	218	239	226
	885	180	180	188	202	219	208
	888	197	200	204	210	227	219
	909	190	194	200	209	240	226
Mean		177.3	179.3	187.7	200.2	219.6	205.2
SD		12.9	14.4	13.5	14.0	16.9	17.2

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
45.9 mg/kg-d	851	152	153	162	179	200	184
	858	168	174	183	195	224	204
	869	177	184	196	214	232	218
	871	167	170	178	194	207	198
	875	166	167	180	195	205	196
	876	182	184	189	204	221	207
	878	188	191	200	219	238	226
	887	190	192	202	221	245	227
	889	180	186	192	206	217	208
	903	178	184	185	196	214	201
Mean		174.8	178.5	186.7	202.3	220.3	206.9
SD		11.6	12.3	11.9	13.1	14.7	13.6

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
151.5 mg/kg-d	848	179	181	190	202	218	205
	861	170	170	173	189	203	187
	879	164	170	180	198	210	194
	884	184	181	193	211	223	218
	886	169	168	181	196	204	199
	898	185	179	198	213	237	230
	908	186	183	198	212	236	223
	910	165	167	175	185	203	208
	915	196	197	208	218	237	223
	917	179	180	191	196	212	209
Mean		177.7	177.6	188.7	202.0	218.3	209.6
SD		10.5	9.2	11.3	11.1	14.2	13.9

Table G-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

14-Day Individual Body Weights (grams)

Table G-2 (continued)

	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13	Day 14
500 mg/kg-d	854	174	163	149	151	185	171
	856	155	155	155	162	199	178
	877	184	184	186	183	195	174
	890	198	198	189	210	248	228
	891	166	166	174	190	216	201
	893	181	176	180	173	208	203
	902	178	159	165	178	217	199
	912	177	180	190	185	208	188
	914	173	168	175	189	216	206
	916	190	192	193	174	219	205
Mean		177.6	174.1	*175.6	*179.5	211.1	195.3
SD		12.0	14.3	15.1	16.2	17.0	17.6

*$\rho \leq 0.05$

APPENDIX H

SUMMARY OF 14-DAY BODY WEIGHT CHANGES
AND INDIVIDUAL DATA

Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

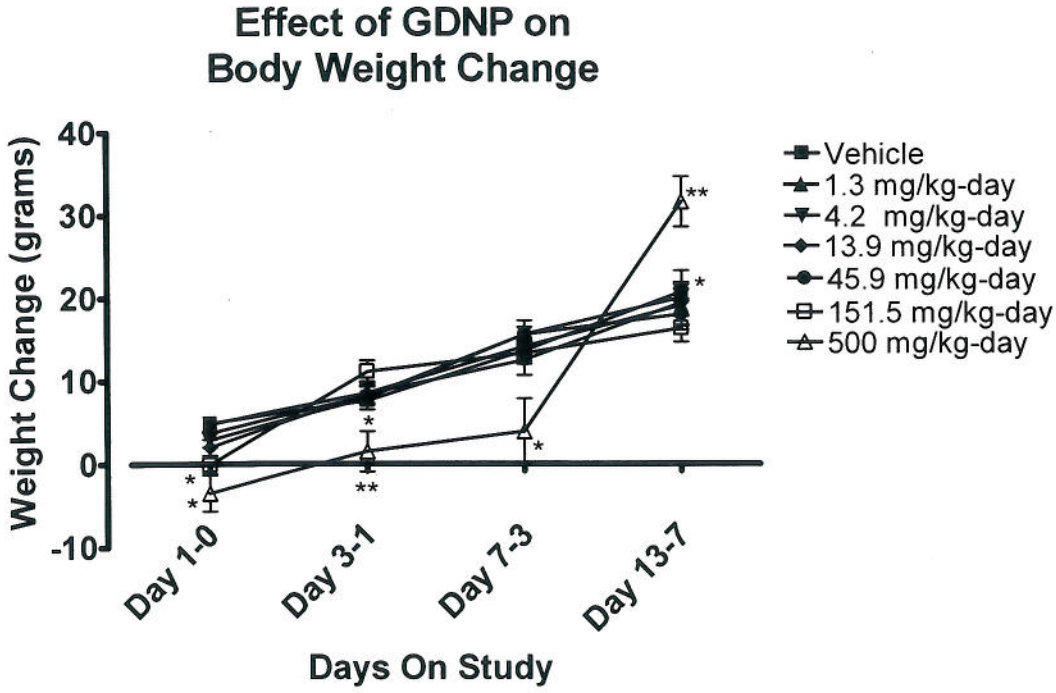


Figure H-1. Summary of 14-Day Body Weight Changes and Individual Data

Note: Rats were fasted overnight prior to necropsy on Day 14. *$\rho \leq 0.05$; **$\rho \leq 0.01$

Table H-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Body Weight Changes (grams)

		GDNP (mg/kg-day)						
Period		**Vehicle Control**	**1.3**	**4.2**	**13.9**	**45.9**	**151.5**	**500**
Day 0-1	**Mean**	4.9	4.8	2.9	***2**	3.7	****-0.1**	****-3.5**
	S.D.	2.7	2.7	2.7	3.1	2.3	3.3	6.9
	N	10	10	10	10	10	10	10
Day 1-3	**Mean**	7.6	8.5	7.9	8.4	8.2	***11.1**	***1.5**
	S.D.	2.2	4.5	4.4	3.4	3.5	4.3	7.6
	N	10	10	10	10	10	10	10
Day 3-7	**Mean**	13.6	14.1	15.6	12.5	15.6	13.3	***3.9**
	S.D.	4.0	3.4	5.2	5.8	2.8	4.1	12.5
	N	10	10	10	10	10	10	10
Day 7-13	**Mean**	20.7	19.1	20.0	19.4	18.0	***16.3**	****31.6**
	S.D.	3.6	8.0	10.3	5.2	5.8	5.2	9.6
	N	10	10	10	10	10	10	10
Net	**Mean**	46.8	46.5	46.4	42.3	45.5	40.6	***33.5**
	S.D.	8.3	8.3	11.8	9.4	8.1	6.7	14.4
	N	10	10	10	10	10	10	10

Bold type indicates value is significantly different from control value
$\rho \leq 0.05$; *$\rho \leq 0.01$**

Table H-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

14-Day Individual Body Weight Changes (grams)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change Day 1-13
Vehicle Control	849	0	6	11	20	-20	37
	853	5	6	11	20	-20	42
	859	4	8	12	21	-12	45
	860	7	12	15	23	-13	57
	863	7	9	17	27	-18	60
	874	5	7	22	21	-11	55
	892	3	8	16	13	-15	40
	899	2	4	12	19	-12	37
	901	8	9	8	20	-16	45
	905	8	7	12	23	2	50
Mean		4.9	7.6	13.6	20.7	-13.5	46.8
SD		2.7	2.2	4.0	3.6	6.4	8.3

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change Day 1-13
1.3 mg/kg-d	852	6	6	14	26	-15	52
	857	0	8	14	13	-16	35
	867	6	5	18	27	-20	56
	870	2	17	10	4	-10	33
	883	3	8	9	24	-14	44
	894	9	10	17	15	-11	51
	896	5	10	17	16	-10	48
	897	7	14	14	18	-15	53
	900	3	2	18	31	-13	54
	907	7	5	10	17	-13	39
Mean		4.8	8.5	14.1	19.1	-13.7	46.5
SD		2.7	4.5	3.4	8.0	3.1	8.3

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Day 1-13
4.2 mg/kg-d	855	4	2	17	7	-17	30
	866	5	14	22	20	-17	61
	868	1	9	13	17	-19	40
	873	1	4	22	10	-4	37
	880	2	12	12	44	-36	70
	895	2	11	19	14	-9	46
	904	4	13	8	19	-16	44
	906	4	5	12	23	-13	44
	911	-2	5	10	27	-17	40
	913	8	4	21	19	-17	52
Mean		2.9	7.9	15.6	20.0	-16.5	46.4
SD		2.7	4.4	5.2	10.3	8.2	11.8

Table H-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

14-Day Individual Body Weight Changes (grams)

Table H-2 (continued)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change Day 1-13
13.9 mg/kg-d	850	4	4	21	23	-21	52
	862	0	10	8	13	-14	31
	864	-1	11	12	19	-16	41
	865	5	6	23	21	-20	55
	872	-4	12	6	19	-11	33
	881	5	9	13	13	-16	40
	882	4	14	13	21	-13	52
	885	0	8	14	17	-11	39
	888	3	4	6	17	-8	30
	909	4	6	9	31	-14	50
Mean		***2.0**	8.4	12.5	19.4	-14.4	42.3
SD		3.1	3.4	5.8	5.2	4.0	9.4

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change Day 1-13
45.9 mg/kg-d	851	1	9	17	21	-16	48
	858	6	9	12	29	-20	56
	869	7	12	18	18	-14	55
	871	3	8	16	13	-9	40
	875	1	13	15	10	-9	39
	876	2	5	15	17	-14	39
	878	3	9	19	19	-12	50
	887	2	10	19	24	-18	55
	889	6	6	14	11	-9	37
	903	6	1	11	18	-13	36
Mean		3.7	8.2	15.6	18.0	-13.4	45.5
SD		2.3	3.5	2.8	5.8	3.8	8.1

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change Day 1-13
151.5 mg/kg-d	848	2	9	12	16	-13	39
	861	0	3	16	14	-16	33
	879	6	10	18	12	-16	46
	884	-3	12	18	12	-5	39
	886	-1	13	15	8	-5	35
	898	-6	19	15	24	-7	52
	908	-3	15	14	24	-13	50
	910	2	8	10	18	5	38
	915	1	11	10	19	-14	41
	917	1	11	5	16	-3	33
Mean		****-0.1**	***11.1**	13.3	***16.3**	-8.7	40.6
SD		3.3	4.3	4.1	5.2	6.8	6.7

Table H-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

14-Day Individual Body Weight Changes (grams)

Table H-2 (continued)

	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Day 13-14	Net Change Day 1-13
500 mg/kg-d	854	-11	-14	2	34	-14	11
	856	0	0	7	37	-21	44
	877	0	2	-3	12	-21	11
	890	0	-9	21	38	-20	50
	891	0	8	16	26	-15	50
	893	-5	4	-7	35	-5	27
	902	-19	6	13	39	-18	39
	912	3	10	-5	23	-20	31
	914	-5	7	14	27	-10	43
	916	2	1	-19	45	-14	29
Mean		****-3.5**	***1.5**	***3.9**	****31.6**	***-15.8**	33.5
SD		6.9	7.6	12.5	9.6	5.3	14.4

Bold type indicates value is significantly different from control value
***$\rho \leq 0.05$; **$\rho \leq 0.01$**

APPENDIX I

SUMMARY OF 14-DAY FOOD CONSUMPTION
AND INDIVIDUAL DATA

Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

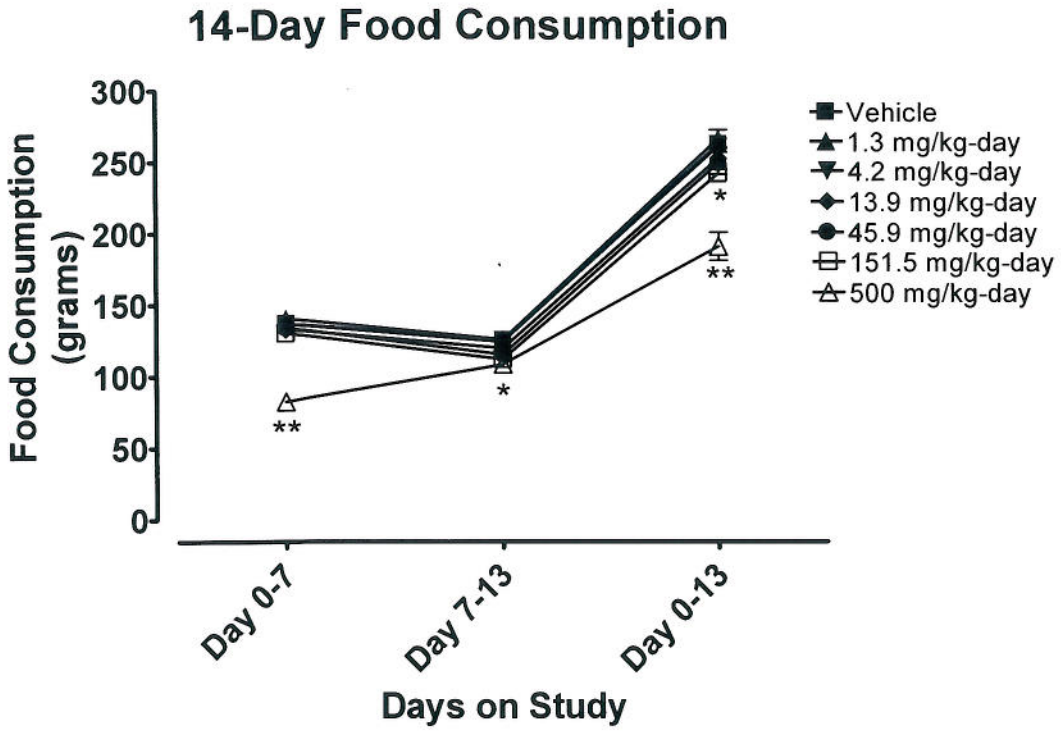


Table I-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Food Consumption (grams)

		GDNP (mg/kg-day)						
Period		**Vehicle Control**	**1.3**	**4.2**	**13.9**	**45.9**	**151.5**	**500**
Day 0-7	**Mean**	136.80	140.3	136.6	132.6	133.7	129.9	****82.3**
	S.D.	6.46	10.9143	8.64356	12.6069	11.7478	8.68524	17.6701
	N	10	10	10	10	10	10	10
Day 7-13	**Mean**	125.3	125.4	124.1	119.5	***115.1**	****111.8**	***108.2**
	S.D.	7.04036	10.5536	10.7025	12.3401	12.9739	11.7454	17.7751
	N	10	10	10	10	10	10	10
TOTAL	**Mean**	262.1	265.7	260.7	252.1	248.8	***241.7**	****190.5**
	S.D.	12.9052	19.9391	16.647	23.8954	24.0823	18.8741	31.0349
	N	10	10	10	10	10	10	10

Bold type indicates value is significantly different from control value
***$\rho \leq 0.05$; **$\rho \leq 0.01$**

Table I-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Food Consumption (grams)

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
Vehicle	849	610	479	586	462	131	124	255
Control	853	578	443	585	459	135	126	261
	859	580	448	620	501	132	119	251
	860	587	445	566	430	142	136	278
	863	572	431	567	433	141	134	275
	874	576	430	564	433	146	131	277
	892	610	473	569	448	137	121	258
	899	603	476	528	414	127	114	241
	901	595	450	551	423	145	128	273
	905	595	463	509	389	132	120	252
Mean		**590.6**	**453.8**	**564.5**	**439.2**	**136.8**	**125.3**	**262.1**
SD		**14.1**	**18.0**	**30.9**	**30.5**	**6.5**	**7.0**	**12.9**

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
1.3 mg/kg-d	852	584	445	551	416	139	135	274
	857	596	472	594	487	124	107	231
	867	598	467	596	474	131	122	253
	870	587	436	608	479	151	129	280
	883	583	458	562	443	125	119	244
	894	598	452	570	444	146	126	272
	896	593	446	563	442	147	121	268
	897	612	460	533	403	152	130	282
	900	603	451	492	346	152	146	298
	907	622	486	528	409	136	119	255
Mean		597.6	457.3	559.7	434.3	140.3	125.4	265.7
SD		12.3	14.7	35.5	42.6	10.9	10.6	19.9

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
4.2 mg/kg-d	855	551	430	549	439	121	110	231
	866	597	454	559	432	143	127	270
	868	592	462	579	463	130	116	246
	873	607	468	585	470	139	115	254
	880	556	424	557	413	132	144	276
	895	589	450	560	442	139	118	257
	904	607	471	508	390	136	118	254
	906	593	463	505	380	130	125	255
	911	576	426	503	367	150	136	286
	913	586	440	540	408	146	132	278
Mean		585.4	448.8	544.5	420.4	136.6	124.1	260.7
SD		19.2	17.7	30.0	34.7	8.6	10.7	16.6

Table I-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Food Consumption (grams)

Table I-2 (continued)

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
13.9 mg/kg-d	850	598	466	564	443	132	121	253
	862	572	460	568	463	112	105	217
	864	576	459	589	481	117	108	225
	865	575	423	581	444	152	137	289
	872	584	455	566	448	129	118	247
	881	579	453	543	427	126	116	242
	882	593	456	553	429	137	124	261
	885	588	455	524	423	133	101	234
	888	582	433	525	391	149	134	283
	909	593	454	506	375	139	131	270
Mean		584.0	451.4	551.9	432.4	132.6	119.5	252.1
SD		8.8	13.1	26.9	31.5	12.6	12.3	23.9

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
45.9 mg/kg-d	851	594	475	592	480	119	112	231
	858	588	446	584	456	142	128	270
	869	604	457	577	451	147	126	273
	871	565	440	532	430	125	102	227
	875	600	474	590	484	126	106	232
	876	564	441	562	459	123	103	226
	878	586	449	544	430	137	114	251
	887	587	434	492	351	153	141	294
	889	588	447	522	406	141	116	257
	903	598	474	539	436	124	103	227
Mean		587.4	453.7	553.4	438.3	133.7	***115.1**	248.8
SD		13.5	15.5	33.2	38.8	11.7	13.0	24.1

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
151.5 mg/kg-d	848	587	459	574	471	128	103	231
	861	601	478	567	469	123	98	221
	879	585	459	569	470	126	99	225
	884	593	460	546	433	133	113	246
	886	578	454	548	443	124	105	229
	898	576	442	558	429	134	129	263
	908	588	456	554	426	132	128	260
	910	604	488	544	440	116	104	220
	915	621	486	535	419	135	116	251
	917	590	442	519	396	148	123	271
Mean		592.3	462.4	551.4	439.6	129.9	****111.8**	***241.7**
SD		13.4	16.4	16.8	24.6	8.7	11.7	18.9

Table I-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Food Consumption (grams)

Table I-2 (continued)

	Animal ID	D0	D7-Pre	D7-Post	D13	Day 0-7	Day 7-13	Total
500 mg/kg-d	854	602	555	543	454	47	89	136
	856	589	512	559	445	77	114	191
	877	594	503	556	481	91	75	166
	890	575	465	560	420	110	140	250
	891	598	503	557	447	95	110	205
	893	574	503	572	470	71	102	173
	902	569	486	573	454	83	119	202
	912	570	492	531	429	78	102	180
	914	595	496	567	448	99	119	218
	916	597	525	527	415	72	112	184
Mean		586.3	504.0	554.5	446.3	****82.3**	***108.2**	****190.5**
SD		12.8	23.9	16.0	20.7	17.7	17.8	31.0

Bold type indicates value is significantly different from control value
***$\rho \leq 0.05$; **$\rho \leq 0.01$**

APPENDIX J
SUMMARY OF 14-DAY CLINICAL CHEMISTRY AND INDIVIDUAL DATA

Table J-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Summary of 14-Day Clinical Chemistry

		Vehicle Control	1.3	4.2	13.9	45.9	151.5	500
ALB (g/dL)	Mean	3.3	3.6	3.6	3.6	**3.8	**4.0	3.6
	S.D.	0.3	0.3	0.3	0.3	0.3	0.2	0.3
ALKP (U/L)	Mean	195.2	249.3	211.4	207.8	172.9	144.2	239.2
	S.D.	44.5	32.3	34.7	37.6	66.6	38.2	82.3
ALT (U/L)	Mean	56.9	53.6	54.3	57.6	65.2	65.5	**96.8
	S.D.	10.2	10.4	11.5	9.8	14.0	12.7	24.9
BUN (mg/dL)	Mean	107.9	95.7	115.5	102.9	**88.3	**87.5	**116.2
	S.D.	38.4	7.6	38.2	15.7	14.4	20.1	38.7
Ca (mg/dL)	Mean	17.4	18.3	19.2	21.3	**26.5	**28.2	**21.6
	S.D.	3.2	3.3	3.4	3.8	8.8	5.1	2.8
CHOL (mg/dL)	Mean	10.8	11.1	10.7	11.1	*11.28	**11.51	**11.8
	S.D.	0.3	0.4	0.7	0.5	0.3	0.4	0.2
CREA (mg/dL)	Mean	57.9	61.1	62.0	64.6	81.1	99.9	**105.9
	S.D.	17.0	18.1	21.8	9.8	23.5	12.0	14.5
GLOB (g/dL)	Mean	0.5	0.5	0.5	0.5	*0.5	**0.51	**0.3
	S.D.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
GLU (mg/dL)	Mean	2.8	2.8	2.9	2.7	3.1	3.4	3.3
	S.D.	0.3	0.1	0.7	0.3	0.3	0.3	0.3
LDH (U/L)	Mean	127.7	120.1	113.7	111.7	116.9	112.5	130.8
	S.D.	26.0	35.2	16.9	23.6	17.1	22.9	18.0
PHOS (mg/dL)	Mean	9.7	10.3	10.4	10.3	9.1	9.2	10.1
	S.D.	1.1	1.1	2.0	1.2	1.2	1.4	1.4
TBIL (mg/dL)	Mean	0.1	0.2	0.2	0.2	**0.3	0.2	0.2
	S.D.	0.0	0.1	0.1	0.1	0.1	0.1	0.1
TP (g/dL)	Mean	6.1	6.4	6.5	6.3	**6.9	**7.3	**6.9
	S.D.	0.3	0.2	0.8	0.4	0.5	0.3	0.4
Na (mmol/L)	Mean	152.9	152.6	152.5	153.0	152.4	153.5	150.3
	S.D.	2.5	1.8	2.9	1.6	1.5	3.0	2.6
K (mmol/L)	Mean	7.3	8.4	7.9	8.0	7.5	7.3	7.3
	S.D.	1.0	1.3	0.8	1.6	1.2	0.7	0.8
Cl (mmol/L)	Mean	106.4	105.7	105.5	104.9	104.9	104.0	101.5
	S.D.	0.8	1.3	1.2	1.3	2.6	1.7	1.0

Bold type indicates value is significantly different from control value

*$\rho \leq 0.05$ **$\rho \leq 0.05$

Table J-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Clinical Chemistry

	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
Vehicle	849	2.9	165	53	116	14	10.6	44	0.5	3.0	133	9.3	0.1	5.9	149	7.0	106
	853	3.4	150	44	91	12	10.9	55	0.5	3.1	104	10.9	0.1	6.5	154	8.6	108
	859	2.8	221	61	204	19	10.2	30	0.5	3.3	92	10.4	0.1	6.1	151	6.9	106
	860	3.6	149	43	84	14	11.2	80	0.4	2.8	159	9.9	0.1	6.5	154	6.2	106
	863	3.5	236	70	136	18	11.0	62	0.5	2.7	153	8.7	0.1	6.2	153	9.1	107
	874	3.5	210	56	98	22	10.7	74	0.5	2.5	123	9.7	0.2	6.0	150	6.7	106
	892	3.5	235	53	98	21	10.8	62	0.5	2.5	103	11.7	0.1	6.0	156	7.2	106
	899	3.4	177	75	99	19	10.7	46	0.5	2.6	105	9.7	0.1	6.0	155	7.7	Not Valid
	901	3.0	141	61	82	17	10.9	43	0.4	2.6	142	7.7	0.1	5.7	156	6.0	Not Valid
	905	3.5	268	53	71	18	11.4	81	0.3	2.5	163	8.9	0.1	5.9	151	7.6	Not Valid
	Mean	3.3	195.2	56.9	107.9	17.4	10.8	57.9	0.5	2.8	127.7	9.7	0.1	6.1	152.9	7.3	106.4
	S.D.	0.3	44.5	10.2	38.4	3.2	0.3	17.0	0.1	0.3	26.0	1.1	0.0	0.3	2.5	1.0	0.8

	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
1.3 mg/kg-d	852	3.1	238	50	98	20	11.1	35	0.6	3.0	76	10.8	0.1	6.1	152	10.3	106
	867	3.5	227	42	91	15	10.8	66	0.4	2.9	111	9.9	0.1	6.5	154	7.3	108
	857	3.3	199	38	86	21	10.8	45	0.5	3.0	121	9.7	0.1	6.3	152	8.0	104
	870	3.6	277	46	107	19	11.3	47	0.6	2.7	93	12.5	0.2	6.3	151	11.0	106
	883	3.7	295	53	87	16	10.9	73	0.4	2.6	151	10.6	0.3	6.3	152	8.1	106
	894	3.7	217	61	97	16	11.2	87	0.5	2.7	109	9.0	0.2	6.4	155	7.1	105
	896	3.6	283	74	109	22	11.2	75	0.5	2.8	150	8.8	0.3	6.4	152	7.2	105
	897	3.9	262	60	93	21	10.8	43	0.6	2.6	89	10.7	0.2	6.4	151	8.9	Not Valid
	900	4.1	225	58	93	12	12.1	82	0.6	2.8	193	11.0	0.3	6.9	156	7.9	Not Valid
	907	3.4	270	54	96	21	11.0	58	0.6	2.8	108	9.6	0.2	6.2	151	7.9	Not Valid
	Mean	3.6	249.3	53.6	95.7	18.3	11.1	61.1	0.5	2.8	120.1	10.3	0.2	6.4	152.6	8.4	105.7
	S.D.	0.3	32.3	10.4	7.6	3.3	0.4	18.1	0.1	0.1	35.2	1.1	0.1	0.2	1.8	1.3	1.3

Table J-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Clinical Chemistry

Table I-2 (continued)

	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
4.2 mg/kg-d	855	3.2	145	57	215	20	10.0	11	0.4	2.8	126	9.8	0.1	5.9	150	6.9	104
	866	3.9	209	56	118	21	10.2	74	0.4	3.2	93	8.8	0.1	7.1	154	7.1	104
	868	3.3	194	44	87	14	9.2	67	0.5	2.9	104	9.4	0.1	6.2	158	8.8	106
	873	3.9	251	51	107	17	10.9	87	0.4	2.4	115	14.3	0.2	6.3	154	8.5	107
	880	3.7	232	84	99	23	11.2	75	0.4	2.7	154	11.2	0.2	6.3	150	8.4	106
	895	3.8	189	55	107	16	11.2	55	0.5	2.6	116	9.3	0.2	6.4	156	6.8	106
	904	3.5	240	49	137	24	10.6	71	0.6	2.4	107	7.0	0.1	5.9	151	8.0	Not Valid
	906	3.1	230	54	94	22	11.0	41	0.5	2.8	103	10.3	0.3	5.9	152	8.5	Not Valid
	911	3.8	177	42	84	16	11.4	65	0.5	4.7	115	11.6	0.1	8.5	151	8.3	Not Valid
	913	3.6	247	51	107	19	11.0	74	0.5	2.3	104	12.1	0.1	6.0	149	7.3	Not Valid
	Mean	3.6	211.4	54.3	115.5	19.2	10.7	62.0	0.5	2.9	113.7	10.4	0.2	6.5	152.5	7.9	105.5
	S.D.	0.3	34.7	11.5	38.2	3.4	0.7	21.8	0.1	0.7	16.9	2.0	0.1	0.8	2.9	0.8	1.2
	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
13.9 mg/kg-d	850	3.4	228	43	113	18	10.8	72	0.4	2.6	96	8.6	0.1	6.0	153	6.6	107
	862	3.0	165	48	94	17	10.7	64	0.5	2.5	103	10.0	0.1	5.5	153	7.3	105
	864	3.4	213	49	108	24	12.2	67	0.5	3.2	153	11.1	0.1	6.7	155	8.1	106
	865	3.6	177	54	124	25	10.6	54	0.7	3.1	74	12.9	0.1	6.7	152	11.6	104
	872	4.1	183	73	108	16	10.9	65	0.3	2.7	144	10.6	0.3	6.7	151	8.0	104
	881	3.6	273	58	87	23	11.6	80	0.5	2.9	109	9.7	0.2	6.6	155	7.2	103
	882	3.9	264	55	74	18	11.6	77	0.5	2.7	126	10.5	0.2	6.6	153	8.3	105
	885	3.7	174	70	117	21	11.2	60	0.5	2.4	98	11.1	0.1	6.1	150	9.5	Not Valid
	888	3.4	190	60	113	25	10.8	50	0.5	2.6	113	9.1	0.1	6.0	154	6.4	Not Valid
	909	3.4	211	66	91	26	10.7	57	0.6	2.7	101	9.3	0.2	6.1	154	7.0	Not Valid

Table J-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Clinical Chemistry

	Mean	3.6	207.8	57.6	102.9	21.3	11.1	64.6	0.5	2.7	111.7	10.3	0.2	6.3	153.0	8.0	104.9
	S.D.	0.3	37.6	9.8	15.7	3.8	0.5	9.8	0.1	0.3	23.6	1.2	0.1	0.4	1.6	1.6	1.3

Table J-2 (continued)

	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
45.9 mg/kg-d	851	3.2	178	40	108	15	11.6	47	0.5	3.0	103	11.9	0.1	6.2	155	9.0	107
	858	3.6	98	53	78	19	10.6	61	0.4	3.0	109	8.7	0.2	6.6	153	6.5	109
	869	3.7	231	68	99	24	11.0	106	0.4	3.4	104	8.6	0.3	7.0	151	6.9	104
	871	4.0	188	76	70	44	11.6	95	0.6	2.9	121	9.5	0.3	6.9	153	9.1	104
	875	4.0	217	60	68	22	11.5	90	0.5	3.2	118	8.2	0.2	7.2	152	6.3	102
	876	3.9	98	85	85	38	11.4	89	0.6	2.8	110	8.9	0.3	6.7	153	7.4	106
	878	4.1	278	61	85	26	11.5	120	0.5	3.6	117	8.1	0.3	7.7	154	6.5	102
	887	3.7	221	70	108	21	11.0	52	0.5	2.8	102	9.1	0.2	6.5	150	7.1	Not Valid
	889	4.1	140	56	84	30	11.5	71	0.5	3.4	160	10.1	0.3	7.5	152	9.4	Not Valid
	903	3.8	80	83	98	26	11.1	80	0.5	2.9	125	8.2	0.4	6.7	151	6.7	Not Valid
	Mean	**3.8**	172.9	65.2	88.3	**26.5**	**11.3**	**81.1**	0.5	**3.1**	116.9	9.1	**0.3**	**6.9**	152.4	7.5	104.9
	S.D.	0.3	66.6	14.0	14.4	8.8	0.3	23.5	0.1	0.3	17.1	1.2	0.1	0.5	1.5	1.2	2.6

	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
151.5 mg/kg-d	848	3.8	166	61	81	24	10.5	120	0.4	3.5	121	7.4	0.1	7.3	157	6.1	106
	861	3.7	102	41	74	26	11.6	83	0.5	3.8	84	10.7	0.1	7.5	154	8.2	105
	879	4.0	177	63	80	30	12.1	99	0.6	3.7	86	11.1	0.1	7.7	153	8.3	102
	884	4.2	178	59	82	27	11.2	106	0.5	3.7	143	8.0	0.1	7.9	155	7.0	102
	886	4.0	101	80	88	39	11.8	113	0.5	3.3	145	9.3	0.3	7.2	152	7.6	104
	898	3.9	206	86	138	26	11.4	92	0.5	2.9	86	11.6	0.1	6.8	149	8.0	105
	908	4.0	138	70	71	27	11.6	98	0.6	3.1	121	9.2	0.3	7.2	149	7.5	Not Valid
	910	3.9	159	73	72	28	11.7	106	0.5	3.3	129	8.0	0.1	7.2	155	7.1	Not Valid
	915	4.2	106	65	85	34	11.9	83	0.5	3.3	105	8.7	0.4	7.5	153	6.7	Not Valid
	917	4.1	109	57	104	21	11.3	99	0.5	3.0	105	8.4	0.3	7.1	158	6.5	Not Valid

Table J-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Clinical Chemistry

	Mean	**4.0**	144.2	65.5	87.5	**28.2**	**11.5**	**99.9**	0.5	**3.4**	112.5	9.2	0.2	**7.3**	153.5	7.3	104.0
	S.D.	0.2	38.2	12.7	20.1	5.1	0.4	12.0	0.1	0.3	22.9	1.4	0.1	0.3	3.0	0.7	1.7

Table J-2 (continued)

	Animal	ALB	ALKP	ALT	AST	BUN	Ca	CHOL	CREA	GLOB	GLU	PHOS	TBIL	TP	Na	K	Cl
500 mg/kg-d	ID	(g/dL)	(U/L)	(U/L)	(U/L)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(g/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(g/dL)	(mmol/L)	(mmol/L)	(mmol/L)
	854	3.1	171	104	244	26	11.3	95	0.3	3.2	128	10.7	0.1	6.3	153	7.4	106
	856	3.4	297	79	91	18	11.9	105	0.2	3.4	122	10.2	0.1	6.8	148	7.4	101
	877	3.1	129	100	215	22	11.7	75	0.3	3.5	150	12.9	0.2	6.7	150	9.1	102
	890	3.8	299	143	96	22	12.1	122	0.5	4.0	138	10.1	0.1	7.8	150	7.2	100
	891	3.6	293	133	122	23	11.9	103	0.4	3.2	117	10.7	0.1	6.7	153	6.7	103
	893	3.2	299	85	114	20	11.4	104	0.3	3.2	122	9.8	0.3	6.4	150	7.4	102
	902	4.0	329	78	100	19	11.9	99	0.4	3.1	135	8.4	0.3	7.0	147	6.9	101
	912	3.9	144	85	106	19	11.9	121	0.3	3.3	124	9.1	0.1	7.2	152	7.5	Not Valid
	914	3.8	132	95	113	26	11.7	105	0.4	3.3	105	8.2	0.1	7.1	155	6.1	Not Valid
	916	3.4	231	73	89	25	11.8	119	0.3	3.0	164	11.1	0.2	6.5	148	7.3	Not Valid
	Mean	3.6	239.2	**96.8**	116.2	**21.6**	**11.8**	**105.9**	**0.3**	**3.3**	130.8	10.1	0.2	**6.9**	150.3	7.3	101.5
	S.D.	0.3	82.3	24.9	38.7	2.8	0.2	14.5	0.1	0.3	18.0	1.4	0.1	0.4	2.6	0.8	1.0

Bold type indicates value is significantly different from control value

APPENDIX K
Summary of 14-Day Hematology and Individual Data

Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Table K-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Hematology

		GDNP (mg/kg-day)						
		Vehicle Control	**1.3**	**4.2**	**13.9**	**45.9**	**151.5**	**500**
WBC (K/μL)	**Mean**	7.1	8.8	8.6	10.2	***10.3**	10.0	****13.2**
	S.D.	3.5	3.8	2.6	4.3	3.2	3.6	4.8
NEU (K/μL)	**Mean**	0.5	0.7	0.5	0.7	0.8	0.7	***2.2**
	S.D.	0.3	0.7	0.2	0.3	0.4	0.5	1.9
(%N)	**Mean**	7.5	7.2	6.7	6.8	7.4	6.4	***15.5**
	S.D.	2.0	3.6	2.9	2.1	3.3	3.0	10.5
LYM (K/μL)	**Mean**	6.1	7.4	7.5	8.9	***8.8**	8.6	***9.4**
	S.D.	2.9	2.8	2.5	3.9	2.7	3.1	3.4
(%L)	**Mean**	85.2	85.1	86.4	86.5	85.8	86.3	****72.1**
	S.D.	3.5	5.3	4.2	2.2	4.2	5.0	10.4
MONO (K/μL)	**Mean**	0.3	0.4	0.3	0.4	0.4	0.4	****1.0**
	S.D.	0.2	0.3	0.1	0.2	0.2	0.2	0.6
(%M)	**Mean**	4.7	4.5	4.3	3.9	4.1	4.1	***7.5**
	S.D.	2.2	1.4	1.5	1.2	1.3	2.1	2.6
EOS (K/μL)	**Mean**	0.1	0.1	0.1	0.1	0.1	0.1	****0.2**
	S.D.	0.0	0.1	0.0	0.1	0.0	0.1	0.1
(%E)	**Mean**	0.9	1.0	0.9	1.0	0.9	0.9	***1.4**
	S.D.	0.3	0.5	0.5	0.6	0.3	0.6	0.6
BASO (K/μL)	**Mean**	0.1	0.2	0.1	0.2	0.2	0.2	****0.5**
	S.D.	0.1	0.2	0.0	0.1	0.1	0.1	0.2
(%B)	**Mean**	1.8	2.2	1.6	1.9	1.8	2.3	****3.5**
	S.D.	0.7	0.9	0.6	0.5	0.4	0.8	0.9
RBC (M/μL)	**Mean**	7.1	7.4	7.2	7.4	7.1	7.1	6.9
	S.D.	0.3	0.3	0.3	0.3	0.2	0.4	0.4
HGB (g/dL)	**Mean**	14.2	14.8	14.5	14.9	14.5	14.1	****13.3**
	S.D.	0.6	0.8	0.7	0.8	0.3	0.5	0.7

*$\rho \leq 0.05$ **$\rho \leq 0.05$

Table K-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Hematology

Table K-1 Continued

HCT	**Mean**	41.0	42.5	41.3	42.5	40.8	40.1	***38.6**
(%)	**S.D.**	1.8	2.2	1.9	2.0	1.1	1.5	2.1
MCV	**Mean**	57.5	57.5	57.2	57.3	57.6	56.4	56.1
(fL)	**S.D.**	1.3	1.4	1.9	1.2	1.0	1.1	1.4
MCH	**Mean**	20.0	20.1	20.0	20.2	20.4	19.8	****19.3**
(pg)	**S.D.**	0.4	0.5	0.8	0.5	0.4	0.4	0.6
MCHC	**Mean**	34.8	34.9	35.0	35.2	35.4	35.1	34.4
(g/dL)	**S.D.**	0.6	0.5	0.4	0.8	0.6	0.5	1.0
RDW	**Mean**	15.0	15.1	15.0	15.3	14.4	15.0	****16.9**
(%)	**S.D.**	0.6	0.6	0.8	0.8	0.6	0.8	1.7
PLT	**Mean**	977.3	1098.6	1043.1	1140.3	1171.4	1149.5	973.8
(K/μL)	**S.D.**	327.3	282.9	381.4	293.7	133.9	177.1	274.6
MPV	**Mean**	4.6	4.4	4.5	4.6	4.6	4.6	4.7
(fL)	**S.D.**	0.3	0.3	0.3	0.3	0.3	0.4	0.5

Bold type indicates value is significantly different from control value
***ρ ≤ 0.05; ****ρ ≤ 0.01**

Table K-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Hematology

	Animal ID	WBC (K/uL)	NEU (K/uL)	(%N)	LYM (K/uL)	(%L)	MONO (K/uL)	(%M)	EOS (K/uL)	(%E)	BASO (K/uL)	(%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
Vehicle	849	13.4	1.0	7.8	11.4	85.7	0.5	4.1	0.1	0.8	0.2	1.7	7.4	14.2	40.9	55.3	19.2	34.8	15.5	1009.0	4.4
	853	4.6	0.3	7.2	4.0	87.2	0.2	3.4	0.0	1.0	0.1	1.2	7.5	15.1	43.3	57.4	20.0	34.9	14.5	957.0	4.4
	859	8.8	0.4	4.5	7.6	85.9	0.6	6.8	0.1	1.1	0.1	1.7	7.3	15.0	42.9	58.9	20.6	35.0	14.9	90.6	4.9
	860	6.3	0.3	4.4	5.6	89.4	0.2	3.9	0.1	0.9	0.1	1.5	7.3	14.4	41.8	57.1	19.7	34.6	16.1	1063.0	4.2
	863	9.3	0.8	8.5	7.7	82.9	0.4	4.7	0.1	1.2	0.3	2.8	7.3	14.3	41.9	57.7	19.7	34.1	15.0	1175.0	4.1
	874	8.9	0.8	8.8	7.1	80.0	0.6	6.7	0.1	1.2	0.3	3.4	7.0	14.1	38.9	55.7	20.1	36.2	15.7	1176.0	4.6
	892	1.4	0.1	8.6	1.1	80.1	0.1	9.1	0.0	1.0	0.0	1.2	7.1	14.1	40.6	57.6	20.0	34.7	15.0	1212.0	4.9
	899	3.9	0.3	6.6	3.5	89.7	0.1	1.9	0.0	0.4	0.1	1.3	7.2	14.4	41.4	57.2	19.9	34.7	14.6	1147.0	4.5
	901	5.2	0.6	10.8	4.4	83.2	0.2	3.7	0.1	1.1	0.1	1.2	6.9	13.9	40.3	58.3	20.1	34.5	13.9	1028.0	4.9
	905	9.3	0.7	7.7	8.2	87.9	0.2	2.4	0.0	0.5	0.1	1.5	6.3	12.9	37.5	59.5	20.5	34.5	14.7	915.0	4.9
	Mean	7.1	0.5	7.5	6.1	85.2	0.3	4.7	0.1	0.9	0.1	1.8	7.1	14.2	41.0	57.5	20.0	34.8	15.0	977.3	4.6
	S.D.	3.5	0.3	2.0	2.9	3.5	0.2	2.2	0.0	0.3	0.1	0.7	0.3	0.6	1.8	1.3	0.4	0.6	0.6	327.3	0.3

	Animal ID	WBC (K/uL)	NEU (K/uL)	(%N)	LYM (K/uL)	(%L)	MONO (K/uL)	(%M)	EOS (K/uL)	(%E)	BASO (K/uL)	(%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
1.3 mg/kg-d	852	8.3	0.3	3.9	7.2	87.7	0.5	5.6	0.0	0.4	0.2	2.4	7.2	14.7	42.4	59.1	20.5	34.7	14.6	1166.0	4.2
	867	5.6	0.5	9.2	4.6	81.8	0.3	5.6	0.1	1.3	0.1	2.2	7.5	15.0	41.8	55.4	19.8	35.8	15.7	1445.0	4.4
	857	13.1	0.7	5.1	11.6	88.0	0.6	4.2	0.1	1.0	0.2	1.7	7.5	15.4	43.6	58.4	20.7	35.5	14.7	1248.0	4.8
	870	6.4	0.3	4.3	5.9	92.0	0.2	2.3	0.0	0.7	0.0	0.7	7.3	14.6	42.4	58.1	20.0	34.5	15.5	395.0	4.8
	883	8.2	0.7	9.0	6.9	84.5	0.3	3.7	0.1	1.2	0.1	1.6	7.3	13.9	40.3	55.5	19.2	34.5	15.6	1255.0	4.5
	894	8.6	0.5	5.9	7.5	87.6	0.3	3.9	0.1	0.7	0.2	2.0	7.2	14.1	41.0	57.0	19.6	34.4	16.2	1301.0	4.3
	896	9.8	0.7	7.2	7.9	80.6	0.7	6.7	0.2	2.1	0.3	3.4	8.0	16.4	47.4	59.3	20.5	34.5	14.6	1052.0	4.2
	897	3.5	0.3	7.6	3.0	85.8	0.1	4.2	0.0	0.6	0.1	1.8	7.0	14.2	40.8	58.6	20.3	34.7	14.2	1037.0	4.9
	900	16.7	2.6	15.7	12.3	73.5	1.0	5.8	0.2	1.1	0.7	3.9	7.9	15.7	44.7	56.7	20.0	35.2	15.0	1063.0	4.3
	907	8.0	0.3	4.0	7.2	89.5	0.2	3.1	0.1	1.3	0.2	2.1	7.1	14.3	40.7	56.9	20.0	35.0	15.1	1024.0	4.2
	Mean	8.8	0.7	7.2	7.4	85.1	0.4	4.5	0.1	1.0	0.2	2.2	7.4	14.8	42.5	57.5	20.1	34.9	15.1	1098.6	4.4
	S.D.	3.8	0.7	3.6	2.8	5.3	0.3	1.4	0.1	0.5	0.2	0.9	0.3	0.8	2.2	1.4	0.5	0.5	0.6	282.9	0.3

Table K-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Hematology

Table K-2 (continued)

	Animal ID	WBC (K/uL)	NEU (K/uL)	(%N)	LYM (K/uL)	(%L)	MONO (K/uL)	(%M)	EOS (K/uL)	(%E)	BASO (K/uL)	(%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
4.2 mg/kg-d	855	5.7	0.4	7.7	4.6	80.6	0.4	7.0	0.1	1.7	0.2	3.0	7.3	13.5	39.1	53.8	18.6	34.6	15.7	1054.0	5.0
	866	7.5	0.5	6.6	6.4	85.2	0.4	5.8	0.1	0.7	0.1	1.7	7.3	14.4	41.1	56.1	19.7	35.1	16.2	1355.0	4.4
	868	10.0	0.5	5.4	9.0	89.8	0.3	2.9	0.0	0.4	0.2	1.5	7.9	15.8	44.7	56.6	20.0	35.4	15.2	1076.0	4.1
	873	12.1	0.5	4.3	11.1	91.1	0.3	2.6	0.1	1.0	0.1	1.1	7.5	14.4	42.1	56.0	19.2	34.3	15.2	877.0	4.7
	880	13.2	1.0	7.5	11.5	87.3	0.4	3.2	0.1	1.0	0.1	1.0	7.3	14.6	41.5	56.8	20.0	35.3	15.5	1161.0	4.7
	895	5.0	0.6	11.1	4.2	83.4	0.2	3.6	0.0	0.6	0.1	1.4	7.1	14.8	42.4	59.4	20.7	34.8	13.5	1298.0	4.5
	904	6.8	0.3	4.3	5.9	87.6	0.4	5.3	0.1	0.9	0.1	1.9	7.4	15.1	43.0	58.3	20.5	35.2	15.2	1102.0	4.2
	906	7.7	0.5	6.2	6.7	86.8	0.4	4.6	0.1	1.2	0.1	1.2	6.9	13.4	38.3	55.7	19.5	35.1	15.5	1143.0	4.3
	911	8.3	0.9	11.4	6.6	80.0	0.4	5.3	0.1	1.5	0.2	1.8	6.8	14.4	40.6	59.5	21.0	35.4	14.0	1327.0	4.4
	913	9.3	0.2	2.5	8.6	92.4	0.3	3.0	0.0	0.3	0.2	1.7	6.8	14.1	40.5	59.4	20.7	34.9	14.2	38.1	4.9
	Mean	8.6	0.5	6.7	7.5	86.4	0.3	4.3	0.1	0.9	0.1	1.6	7.2	14.5	41.3	57.2	20.0	35.0	15.0	1043.1	4.5
	S.D.	2.6	0.2	2.9	2.5	4.2	0.1	1.5	0.0	0.5	0.0	0.6	0.3	0.7	1.9	1.9	0.8	0.4	0.8	381.4	0.3

	Animal ID	WBC (K/uL)	NEU (K/uL)	(%N)	LYM (K/uL)	(%L)	MONO (K/uL)	(%M)	EOS (K/uL)	(%E)	BASO (K/uL)	(%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
13.9 mg/kg-d	850	9.7	0.9	9.2	8.1	84.0	0.5	4.8	0.1	0.7	0.1	1.3	7.3	14.9	42.3	57.8	20.3	35.1	14.0	1207.0	4.5
	862	9.2	0.4	4.7	8.0	87.1	0.4	4.1	0.2	2.3	0.2	1.8	7.3	14.8	41.1	56.0	20.2	36.0	17.1	1025.0	4.9
	864	17.2	1.2	6.8	14.7	85.5	0.9	5.0	0.3	1.6	0.2	1.2	7.6	16.2	44.2	58.1	21.3	36.7	15.7	797.0	4.5
	865	16.8	0.7	3.9	15.3	91.2	0.5	3.0	0.1	0.4	0.2	1.5	7.7	15.9	45.2	58.7	20.7	35.3	16.0	1810.0	5.2
	872	6.6	0.6	8.7	5.7	85.1	0.2	3.3	0.1	0.8	0.1	2.2	7.5	14.8	41.8	55.5	19.6	35.3	15.0	875.0	4.0
	881	8.4	0.4	4.4	7.4	88.1	0.4	4.4	0.1	0.9	0.2	2.2	7.6	15.3	43.3	57.2	20.2	35.3	14.7	1234.0	4.6
	882	12.5	0.8	6.7	10.8	86.1	0.6	4.7	0.1	0.8	0.2	1.8	7.6	14.8	42.6	56.1	19.5	34.7	15.0	1333.0	4.6
	885	11.6	0.7	5.9	10.1	86.7	0.5	4.6	0.1	0.7	0.3	2.1	7.5	15.0	43.3	57.9	20.0	34.6	15.2	1127.0	4.5
	888	4.7	0.4	8.1	3.9	83.6	0.2	4.6	0.0	0.9	0.1	2.8	7.3	14.4	42.8	58.8	19.9	33.8	14.9	865.0	4.5
	909	5.8	0.6	9.7	5.1	87.2	0.1	0.9	0.0	0.4	0.1	1.8	6.7	13.3	38.0	56.6	19.8	34.9	15.0	1130.0	4.7
	Mean	10.2	0.7	6.8	8.9	86.5	0.4	3.9	0.1	1.0	0.2	1.9	7.4	14.9	42.5	57.3	20.2	35.2	15.3	1140.3	4.6
	S.D.	4.3	0.3	2.1	3.9	2.2	0.2	1.2	0.1	0.6	0.1	0.5	0.3	0.8	2.0	1.2	0.5	0.8	0.8	293.7	0.3

Table K-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Hematology

Table K-2 (continued)

	Animal	WBC	NEU		LYM		MONO		EOS		BASO		RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
	ID	(K/uL)	(K/uL)	(%N)	(K/uL)	(%L)	(K/uL)	(%M)	(K/uL)	(%E)	(K/uL)	(%B)	(M/uL)	(g/dL)	(%)	(fL)	(pg)	(g/dL)	(%)	(K/uL)	(fL)
45.9 mg/kg-d	851	9.0	0.4	4.1	7.9	88.2	0.5	5.6	0.1	0.7	0.1	1.4	7.1	14.5	40.7	57.5	20.4	35.5	14.9	1313.0	4.7
	858	5.6	0.3	5.7	5.0	89.4	0.2	2.8	0.0	0.4	0.1	1.7	6.9	14.4	39.2	57.2	21.0	36.6	15.1	1277.0	4.7
	869	12.6	1.1	8.9	10.4	83.0	0.7	5.5	0.1	1.0	0.2	1.7	7.3	14.8	41.7	57.0	20.2	35.4	14.6	1341.0	4.7
	871	12.5	0.5	4.1	11.3	90.4	0.4	3.2	0.1	0.7	0.2	1.8	6.9	14.3	39.9	57.6	20.7	35.9	14.5	1293.0	4.8
	875	7.2	0.9	13.1	5.7	79.3	0.3	4.5	0.1	1.2	0.1	1.9	7.0	14.0	40.5	58.1	20.0	34.5	13.8	1222.0	4.3
	876	9.3	0.8	8.4	7.7	82.6	0.5	5.9	0.1	1.0	0.2	2.1	7.2	14.5	41.7	58.2	20.3	34.8	13.5	1004.0	4.6
	878	13.3	0.5	4.0	11.8	88.6	0.6	4.2	0.1	0.8	0.3	2.4	7.1	14.5	41.2	58.0	20.4	35.2	14.0	983.0	5.0
	887	13.9	1.7	12.6	11.0	79.7	0.6	4.1	0.2	1.3	0.3	2.3	7.3	15.0	42.0	57.4	20.5	35.6	15.5	1061.0	4.1
	889	13.1	0.8	6.0	11.6	88.9	0.3	2.6	0.1	1.0	0.2	1.7	7.1	14.7	42.1	59.4	20.8	35.1	14.2	1131.0	4.5
	903	6.3	0.5	7.4	5.6	88.2	0.2	2.7	0.0	0.7	0.1	1.1	7.0	13.9	39.0	55.6	19.8	35.5	14.3	1089.0	4.2
	Mean	**10.3**	0.8	7.4	**8.8**	85.8	0.4	4.1	0.1	0.9	0.2	1.8	7.1	14.5	40.8	57.6	20.4	35.4	14.4	1171.4	4.6
	S.D.	3.2	0.4	3.3	2.7	4.2	0.2	1.3	0.0	0.3	0.1	0.4	0.2	0.3	1.1	1.0	0.4	0.6	0.6	133.9	0.3

	Animal	WBC	NEU		LYM		MONO		EOS		BASO		RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
151.5 mg/kg-d	ID	(K/uL)	(K/uL)	(%N)	(K/uL)	(%L)	(K/uL)	(%M)	(K/uL)	(%E)	(K/uL)	(%B)	(M/uL)	(g/dL)	(%)	(fL)	(pg)	(g/dL)	(%)	(K/uL)	(fL)
	848	4.6	0.3	7.1	3.9	85.0	0.2	5.4	0.0	0.7	0.1	1.8	7.4	14.5	41.0	55.1	19.4	35.3	15.3	1137.0	4.4
	861	15.4	2.0	12.8	12.4	80.7	0.5	3.2	0.1	0.7	0.4	2.5	7.6	15.1	41.9	55.1	19.9	36.1	15.2	1409.0	4.4
	879	11.5	0.5	4.5	10.2	88.5	0.5	4.3	0.1	0.4	0.3	2.3	7.2	14.5	40.8	57.1	20.2	35.4	15.9	1338.0	5.2
	884	4.6	0.4	8.7	3.9	84.4	0.2	4.3	0.0	0.3	0.1	2.4	7.0	13.8	39.7	57.2	19.9	34.8	14.3	1235.0	4.2
	886	10.8	0.5	4.3	9.3	86.3	0.6	5.3	0.1	1.1	0.3	3.0	6.8	13.6	38.3	56.2	19.9	35.4	13.4	1237.0	4.5
	898	10.2	0.9	8.6	7.9	77.3	0.9	8.9	0.1	1.2	0.4	3.9	6.7	13.6	38.6	58.1	20.4	35.2	15.8	1103.0	4.8
	908	10.5	0.7	6.4	8.9	84.7	0.4	3.6	0.2	2.4	0.3	3.0	7.5	14.4	41.5	55.6	19.3	34.7	15.9	1158.0	3.9
	910	9.4	0.4	4.0	8.6	91.5	0.2	2.4	0.1	0.7	0.1	1.5	6.7	13.4	38.4	57.7	20.1	34.8	14.9	1148.0	4.8
	915	14.7	0.8	5.4	13.2	90.2	0.3	2.2	0.1	0.7	0.2	1.5	7.0	13.8	39.1	56.2	19.9	35.3	14.8	871.0	4.8
	917	8.0	0.2	2.3	7.5	94.0	0.1	1.8	0.1	0.7	0.1	1.2	7.5	14.3	41.8	55.6	19.0	34.1	14.7	859.0	4.7
	Mean	10.0	0.7	6.4	8.6	86.3	0.4	4.1	0.1	0.9	0.2	2.3	7.1	14.1	40.1	56.4	19.8	35.1	15.0	1149.5	4.6
	S.D.	3.6	0.5	3.0	3.1	5.0	0.2	2.1	0.1	0.6	0.1	0.8	0.4	0.5	1.5	1.1	0.4	0.5	0.8	177.1	0.4

Table K-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Individual 14-Day Hematology

Table K-2 (continued)

500 mg/kg-d	Animal ID	WBC (K/uL)	NEU (K/uL)	(%N)	LYM (K/uL)	(%L)	MONO (K/uL)	(%M)	EOS (K/uL)	(%E)	BASO (K/uL)	(%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
	854	8.8	1.1	12.1	6.8	77.8	0.6	6.9	0.1	1.3	0.2	1.9	7.4	13.7	40.5	54.9	18.5	33.8	17.8	1143.0	4.5
	856	18.4	2.2	12.1	13.6	74.2	1.9	10.2	0.1	0.8	0.5	2.7	6.7	13.0	37.5	56.3	19.5	34.6	16.9	1420.0	4.6
	877	11.4	0.7	6.4	8.9	77.5	1.2	10.7	0.3	2.2	0.4	3.2	7.5	13.4	39.9	53.5	17.9	33.5	18.0	472.0	4.4
	890	16.5	1.0	5.9	13.0	79.2	1.6	9.8	0.3	2.1	0.5	3.0	7.1	13.5	40.2	56.6	19.0	33.6	17.0	878.0	5.7
	891	7.0	0.8	11.2	5.3	74.6	0.5	7.1	0.1	1.7	0.4	5.4	7.3	14.2	42.0	57.5	19.4	33.8	16.9	909.0	4.7
	893	15.5	5.3	34.1	8.3	53.7	1.3	8.2	0.2	1.2	0.4	2.9	6.4	12.3	35.9	56.3	19.3	34.3	16.2	1086.0	4.7
	902	18.2	2.3	12.7	14.0	77.1	0.8	4.5	0.3	1.6	0.8	4.2	7.1	14.3	38.9	54.6	20.1	36.9	20.3	887.0	4.9
	912	6.9	0.9	13.7	5.4	78.7	0.2	3.3	0.0	0.3	0.3	3.9	6.7	13.1	38.8	58.0	19.6	33.8	14.6	1200.0	4.8
	914	8.3	0.9	11.0	6.6	79.2	0.5	5.8	0.1	1.4	0.2	2.6	6.9	13.4	39.0	56.6	19.5	34.4	14.9	773.0	4.8
	916	16.8	5.5	32.6	9.2	54.5	1.4	8.1	0.2	1.0	0.6	3.7	6.4	12.2	35.5	55.2	19.0	34.4	17.0	1139.0	3.7
	Mean	**13.2**	**2.2**	**15.5**	**9.4**	**72.1**	**1.0**	**7.5**	**0.2**	**1.4**	**0.5**	**3.5**	6.9	**13.3**	**38.6**	56.1	**19.3**	34.4	**16.9**	973.8	4.7
	S.D.	4.8	1.9	10.5	3.4	10.4	0.6	2.6	0.1	0.6	0.2	0.9	0.4	0.7	2.1	1.4	0.6	1.0	1.7	274.6	0.5

Bold type indicates value is significantly different from control value

APPENDIX L
SUMMARY OF 14-DAY ORGAN WEIGHTS AND INDIVIDUAL DATA
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Table L-1
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Organ Weights

		GDNP (mg/kg-day)						
Organ	Wt grams	Vehicle Control	1.3	4.2	13.9	45.9	151.5	500
Brain	**Mean**	1.82	1.85	1.78	1.83	1.82	1.77	1.79
	S.D.	0.07	0.09	0.07	0.08	0.09	0.10	0.07
	N	10	10	10	10	10	10	10
Heart	**Mean**	0.84	0.88	0.90	0.88	0.80	0.78	0.80
	S.D.	0.10	0.10	0.13	0.06	0.10	0.11	0.07
	N	10	10	10	10	10	10	10
Kidney	**Mean**	1.78	1.82	1.80	1.83	1.57	1.72	2.55
	S.D.	0.13	0.19	0.13	0.15	0.21	0.13	1.87
	N	10	10	10	10	10	10	10
Liver	**Mean**	7.71	7.51	7.61	7.32	7.51	8.68	****10.55**
	S.D.	1.30	0.67	0.83	0.84	0.77	1.18	1.45
	N	10	10	10	10	10	10	10
Ovaries	**Mean**	0.14	0.15	0.17	****0.17**	0.16	0.16	0.14
	S.D.	0.03	0.02	0.02	0.02	0.02	0.03	0.02
	N	10	10	10	10	10	10	10
Spleen	**Mean**	0.48	0.49	0.50	0.51	0.43	0.45	***0.59**
	S.D.	0.07	0.08	0.07	0.06	0.06	0.05	0.13
	N	10	10	10	10	10	10	10
Uterus	**Mean**	0.59	***0.44**	***0.46**	***0.46**	0.53	***0.47**	****0.37**
	S.D.	0.12	0.10	0.07	0.10	0.14	0.11	0.10
	N	10	10	10	10	10	10	10

Bold type indicates value is significantly different from control value
***$\rho \leq 0.05$; **$\rho \leq 0.01$**

Table L-2
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Normalized Organ/Body Weight Ratios

		GDNP (mg/kg-day)						
Organ		**Vehicle Control**	**1.3**	**4.2**	**13.9**	**45.9**	**151.5**	**500**
Brain	**Mean**	0.87	0.89	0.87	0.90	0.88	0.85	0.86
	S.D.	0.07	0.04	0.06	0.06	0.06	0.08	0.07
	N	10	10	10	10	10	10	10
Heart	**Mean**	0.40	0.42	0.44	0.43	0.39	0.37	0.38
	S.D.	0.03	0.05	0.05	0.03	0.05	0.04	0.03
	N	10	10	10	10	10	10	10
Kidney	**Mean**	0.85	0.87	0.87	0.89	0.76	0.82	1.23
	S.D.	0.10	0.06	0.06	0.05	0.11	0.04	0.96
	N	10	10	10	10	10	10	10
Liver	**Mean**	3.64	3.59	3.68	3.56	3.63	***4.14**	****5.04**
	S.D.	0.38	0.18	0.26	0.23	0.26	0.42	0.70
	N	10	10	10	10	10	10	10
Ovaries	**Mean**	0.07	0.07	***0.08**	****0.08**	0.08	****0.08**	0.06
	S.D.	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	N	10	10	10	10	10	10	10
Spleen	**Mean**	0.23	0.23	0.24	0.25	0.21	0.21	****0.28**
	S.D.	0.02	0.03	0.03	0.02	0.02	0.02	0.06
	N	10	10	10	10	10	10	10
Uterus	**Mean**	0.28	0.21	0.22	0.22	0.26	0.23	****0.18**
	S.D.	0.06	0.06	0.03	0.05	0.07	0.06	0.04
	N	10	10	10	10	10	10	10

Bold type indicates value is significantly different from control value
***$p \leq 0.05$; **$p \leq 0.01$**

Table L-3
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats

Summary of 14-Day Normalized Organ/Brain Weight Ratios

		GDNP (mg/kg-day)						
Organ		Vehicle Control	1.3	4.2	13.9	45.9	151.5	500
Heart	Mean	4.64	4.74	5.09	4.83	4.41	4.42	4.45
	S.D.	0.55	0.53	0.82	0.26	0.54	0.65	0.34
	N	10	10	10	10	10	10	10
Kidney	Mean	0.98	0.99	1.01	1.00	0.87	0.97	1.44
	S.D.	0.06	0.08	0.07	0.09	0.11	0.08	1.10
	N	10	10	10	10	10	10	10
Liver	Mean	4.24	4.06	4.28	4.00	4.14	4.93	**5.90
	S.D.	0.67	0.23	0.48	0.41	0.36	0.83	0.76
	N	10	10	10	10	10	10	10
Ovaries	Mean	7.70	8.34	*9.29	**9.34	8.78	*9.21	7.55
	S.D.	1.51	0.90	1.36	0.93	1.14	1.61	1.28
	N	10	10	10	10	10	10	10
Spleen	Mean	2.65	2.66	2.78	2.77	*2.37	2.53	*3.29
	S.D.	0.31	0.40	0.39	0.33	0.26	0.25	0.73
	N	10	10	10	10	10	10	10
Uterus	Mean	3.23	*2.42	*2.58	*2.49	2.95	*2.65	**2.08
	S.D.	0.67	0.62	0.36	0.57	0.86	0.52	0.53
	N	10	10	10	10	10	10	10

Bold type indicates value is significantly different from control value
***$\rho \leq 0.05$; **$\rho \leq 0.01$**

Table L-4
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Raw Organ Weights

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
0	849	1.75	0.65	1.81	6.11	0.07	0.4	0.57
0	853	1.82	0.75	1.93	6.73	0.17	0.45	0.5
0	859	1.75	0.86	1.72	7.21	0.16	0.46	0.46
0	860	1.81	1	1.93	9.27	0.13	0.54	0.67
0	863	1.92	0.8	1.83	8	0.14	0.46	0.77
0	874	1.94	0.93	1.93	8.47	0.16	0.63	0.47
0	892	1.79	0.81	1.65	7	0.14	0.42	0.43
0	899	1.76	0.81	1.6	6.8	0.15	0.45	0.75
0	901	1.81	0.96	1.66	7.2	0.15	0.53	0.67
0	905	1.82	0.87	1.72	10.3	0.13	0.49	0.58
	Avg	1.82	0.84	1.78	7.71	0.14	0.48	0.59
	Std	0.07	0.10	0.13	1.30	0.03	0.07	0.12

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
1.3	852	1.89	0.77	1.69	8.01	0.14	0.58	0.35
1.3	857	1.71	0.95	1.59	6.09	0.16	0.52	0.45
1.3	867	1.71	0.75	1.82	7.18	0.13	0.4	0.65
1.3	870	1.85	0.77	1.55	7.25	0.16	0.42	0.44
1.3	883	1.81	0.78	1.69	7.03	0.14	0.42	0.56
1.3	894	1.95	1.01	2.13	8.05	0.15	0.55	0.41
1.3	896	1.81	0.86	1.87	7.28	0.13	0.52	0.42
1.3	897	1.96	0.88	2	8.11	0.18	0.53	0.45
1.3	900	1.9	0.98	1.97	8.28	0.18	0.6	0.34
1.3	907	1.87	1	1.93	7.79	0.17	0.37	0.36
	Avg	1.85	0.88	1.82	7.51	0.15	0.49	**0.44**
	Std	0.09	0.10	0.19	0.67	0.02	0.08	0.10

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
4.2	855	1.76	0.81	1.65	6.69	0.17	0.35	0.46
4.2	866	1.88	0.78	1.91	8.85	0.15	0.52	0.4
4.2	868	1.8	0.87	1.89	6.69	0.17	0.55	0.43
4.2	873	1.77	0.92	1.77	7.49	0.18	0.51	0.52
4.2	880	1.81	0.75	1.67	6.91	0.13	0.43	0.46
4.2	895	1.85	0.8	1.8	7.98	0.16	0.52	0.58
4.2	904	1.67	0.94	1.56	7.37	0.14	0.43	0.38
4.2	906	1.81	1.19	1.92	6.98	0.16	0.52	0.38
4.2	911	1.66	0.99	1.9	8.4	0.19	0.52	0.46
4.2	913	1.79	0.98	1.94	8.72	0.2	0.6	0.52
	Avg	1.78	0.90	1.80	7.61	0.17	0.50	**0.46**
	Std	0.07	0.13	0.13	0.83	0.02	0.07	0.07

Table L-4
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Raw Organ Weights

Table L-4 continued

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
13.9	850	1.89	0.92	1.76	7.75	0.18	0.54	0.54
13.9	862	1.7	0.82	1.6	5.65	0.16	0.42	0.42
13.9	864	1.79	0.79	1.6	6.3	0.14	0.5	0.37
13.9	865	1.92	0.92	1.73	7.72	0.18	0.49	0.44
13.9	872	1.77	0.89	1.84	7.56	0.2	0.43	0.7
13.9	881	1.92	0.89	1.87	6.67	0.17	0.46	0.42
13.9	882	1.82	0.92	1.93	7.49	0.18	0.56	0.47
13.9	885	1.75	0.78	2.04	7.83	0.15	0.6	0.38
13.9	888	1.84	0.95	2	7.86	0.18	0.55	0.37
13.9	909	1.91	0.96	1.93	8.41	0.17	0.52	0.45
	Avg	1.83	0.88	1.83	7.32	**0.17**	0.51	**0.46**
	Std	0.08	0.06	0.15	0.84	0.02	0.06	0.10

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
45.9	851	1.75	0.94	1.6	6.5	0.13	0.41	0.54
45.9	858	1.75	0.78	1.5	7.32	0.18	0.39	0.79
45.9	869	1.86	0.75	1.81	7.29	0.18	0.47	0.64
45.9	871	1.74	0.72	1.55	6.76	0.13	0.34	0.43
45.9	875	1.97	0.78	1.62	7.57	0.15	0.49	0.45
45.9	876	1.79	0.7	1.47	6.81	0.16	0.37	0.56
45.9	878	1.96	0.8	1.79	8.85	0.15	0.45	0.38
45.9	887	1.86	0.99	1.09	8.15	0.17	0.53	0.37
45.9	889	1.77	0.83	1.73	8.5	0.18	0.46	0.46
45.9	903	1.7	0.71	1.54	7.34	0.16	0.4	0.68
	Avg	1.82	0.80	1.57	7.51	0.16	0.43	0.53
	Std	0.09	0.10	0.21	0.77	0.02	0.06	0.14

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
151.5	848	1.72	0.73	1.5	7.22	0.1	0.4	0.56
151.5	861	1.79	0.73	1.62	7.14	0.16	0.48	0.48
151.5	879	1.89	0.69	1.65	8.05	0.15	0.43	0.66
151.5	884	1.72	0.84	1.78	8.86	0.19	0.48	0.46
151.5	886	1.73	0.62	1.64	7.67	0.14	0.39	0.34
151.5	898	1.82	0.95	1.87	9.2	0.19	0.51	0.47
151.5	908	1.76	0.75	1.8	9.55	0.17	0.43	0.42
151.5	910	1.55	0.8	1.68	10.47	0.16	0.41	0.37
151.5	915	1.86	0.97	1.94	10.17	0.2	0.53	0.59
151.5	917	1.86	0.72	1.73	8.5	0.17	0.41	0.36
	Avg	1.77	0.78	1.72	8.68	0.16	0.45	**0.47**
	Std	0.10	0.11	0.13	1.18	0.03	0.05	**0.11**

Table L-4
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Raw Organ Weights

Table L-4 continued

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
500.0	854	1.71	0.74	1.68	7.6	0.11	0.48	0.39
500.0	856	1.88	0.82	1.85	10.1	0.13	0.45	0.36
500.0	877	1.74	0.64	1.83	8.75	0.1	0.44	0.2
500.0	890	1.87	0.85	2.2	11.9	0.16	0.79	0.47
500.0	891	1.72	0.73	7.86	12.22	0.1	0.532	0.24
500.0	893	1.84	0.88	2.18	11.16	0.13	0.63	0.44
500.0	902	1.73	0.78	1.87	10.27	0.15	0.62	0.38
500.0	912	1.85	0.84	2.09	11.66	0.16	0.49	0.32
500.0	914	1.73	0.84	1.98	10.51	0.15	0.62	0.49
500.0	916	1.79	0.84	1.91	11.33	0.16	0.82	0.43
	Avg	1.79	0.80	2.55	**10.55**	0.14	**0.59**	**0.37**
	Std	0.07	0.07	1.87	1.45	0.02	0.13	0.10

Bold type indicates value is significantly different from control value

Table L-5
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Normalized Organ/Body Weight Ratios

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
0	849	0.99	0.37	1.03	3.47	0.04	0.23	0.32
0	853	0.97	0.40	1.03	3.60	0.09	0.24	0.27
0	859	0.83	0.41	0.81	3.40	0.08	0.22	0.22
0	860	0.75	0.42	0.80	3.86	0.05	0.23	0.28
0	863	0.87	0.36	0.83	3.62	0.06	0.21	0.35
0	874	0.84	0.40	0.84	3.67	0.07	0.27	0.20
0	892	0.90	0.41	0.83	3.52	0.07	0.21	0.22
0	899	0.83	0.38	0.76	3.22	0.07	0.21	0.36
0	901	0.87	0.46	0.79	3.44	0.07	0.25	0.32
0	905	0.81	0.39	0.77	4.60	0.06	0.22	0.26
	Avg	0.87	0.40	0.85	3.64	0.07	0.23	0.28
	Std	0.07	0.03	0.10	0.38	0.01	0.02	0.06

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
1.3	852	0.91	0.37	0.82	3.87	0.07	0.28	0.17
1.3	857	0.90	0.50	0.84	3.22	0.08	0.28	0.24
1.3	867	0.87	0.38	0.92	3.64	0.07	0.20	0.33
1.3	870	0.91	0.38	0.76	3.55	0.08	0.21	0.22
1.3	883	0.96	0.41	0.89	3.72	0.07	0.22	0.30
1.3	894	0.88	0.46	0.96	3.64	0.07	0.25	0.19
1.3	896	0.84	0.40	0.87	3.39	0.06	0.24	0.20
1.3	897	0.86	0.38	0.87	3.54	0.08	0.23	0.20
1.3	900	0.84	0.44	0.88	3.68	0.08	0.27	0.15
1.3	907	0.88	0.47	0.91	3.67	0.08	0.17	0.17
	Avg	0.89	0.42	0.87	3.59	0.07	0.23	0.21
	Std	0.04	0.05	0.06	0.18	0.01	0.03	0.06

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
4.2	855	0.92	0.42	0.86	3.48	0.09	0.18	0.24
4.2	866	0.86	0.36	0.87	4.04	0.07	0.24	0.18
4.2	868	0.97	0.47	1.02	3.60	0.09	0.30	0.23
4.2	873	0.89	0.46	0.89	3.76	0.09	0.26	0.26
4.2	880	0.93	0.39	0.86	3.56	0.07	0.22	0.24
4.2	895	0.88	0.38	0.86	3.80	0.08	0.25	0.28
4.2	904	0.81	0.46	0.76	3.58	0.07	0.21	0.18
4.2	906	0.82	0.54	0.86	3.14	0.07	0.23	0.17
4.2	911	0.78	0.46	0.89	3.93	0.09	0.24	0.21
4.2	913	0.81	0.44	0.87	3.93	0.09	0.27	0.23
	Avg	0.87	0.44	0.87	3.68	***0.08**	0.24	0.22
	Std	0.06	0.05	0.06	0.26	0.01	0.03	0.03

Table L-5
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Normalized Organ/Body Weight Ratios

Table L-5 continued

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
13.9	850	0.91	0.44	0.85	3.74	0.09	0.26	0.26
13.9	862	1.01	0.49	0.95	3.34	0.09	0.25	0.25
13.9	864	0.94	0.42	0.84	3.32	0.07	0.26	0.19
13.9	865	0.94	0.45	0.84	3.77	0.09	0.24	0.21
13.9	872	0.89	0.45	0.93	3.82	0.10	0.22	0.35
13.9	881	0.94	0.44	0.92	3.27	0.08	0.23	0.21
13.9	882	0.81	0.41	0.85	3.31	0.08	0.25	0.21
13.9	885	0.84	0.38	0.98	3.76	0.07	0.29	0.18
13.9	888	0.84	0.43	0.91	3.59	0.08	0.25	0.17
13.9	909	0.85	0.42	0.85	3.72	0.08	0.23	0.20
	Avg	0.90	0.43	0.89	3.56	**0.08	0.25	0.22
	Std	0.06	0.03	0.05	0.23	0.01	0.02	0.05

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
45.9	851	0.95	0.51	0.87	3.53	0.07	0.22	0.29
45.9	858	0.86	0.38	0.74	3.59	0.09	0.19	0.39
45.9	869	0.85	0.34	0.83	3.34	0.08	0.22	0.29
45.9	871	0.88	0.36	0.78	3.41	0.07	0.17	0.22
45.9	875	1.01	0.40	0.83	3.86	0.08	0.25	0.23
45.9	876	0.86	0.34	0.71	3.29	0.08	0.18	0.27
45.9	878	0.87	0.35	0.79	3.92	0.07	0.20	0.17
45.9	887	0.82	0.44	0.48	3.59	0.07	0.23	0.16
45.9	889	0.85	0.40	0.83	4.09	0.09	0.22	0.22
45.9	903	0.85	0.35	0.77	3.65	0.08	0.20	0.34
	Avg	0.88	0.39	0.76	3.63	0.08	0.21	0.26
	Std	0.06	0.05	0.11	0.26	0.01	0.02	0.07

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
151.5	848	0.84	0.36	0.73	3.52	0.05	0.20	0.27
151.5	861	0.96	0.39	0.87	3.82	0.09	0.26	0.26
151.5	879	0.97	0.36	0.85	4.15	0.08	0.22	0.34
151.5	884	0.79	0.39	0.82	4.06	0.09	0.22	0.21
151.5	886	0.87	0.31	0.82	3.85	0.07	0.20	0.17
151.5	898	0.79	0.41	0.81	4.00	0.08	0.22	0.20
151.5	908	0.79	0.34	0.81	4.28	0.08	0.19	0.19
151.5	910	0.75	0.38	0.81	5.03	0.08	0.20	0.18
151.5	915	0.83	0.43	0.87	4.56	0.09	0.24	0.26
151.5	917	0.89	0.34	0.83	4.07	0.08	0.20	0.17
	Avg	0.85	0.37	0.82	*4.14	**0.08	0.21	0.23
	Std	0.08	0.04	0.04	0.42	0.01	0.02	0.06

Table L-5
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Normalized Organ/Body Weight Ratios

Table L-5 continued

mg/kg-day	Rat#	Brain	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
500.0	854	0.83	0.36	0.82	3.71	0.05	0.23	0.19
500.0	856	1.01	0.44	0.99	5.40	0.07	0.24	0.19
500.0	877	0.90	0.33	0.94	4.51	0.05	0.23	0.10
500.0	890	0.86	0.39	1.01	5.46	0.07	0.36	0.22
500.0	891	0.86	0.37	3.95	6.14	0.05	0.27	0.12
500.0	893	0.80	0.38	0.95	4.85	0.06	0.27	0.19
500.0	902	0.78	0.35	0.84	4.61	0.07	0.28	0.17
500.0	912	0.89	0.40	1.00	5.61	0.08	0.24	0.15
500.0	914	0.78	0.38	0.89	4.71	0.07	0.28	0.22
500.0	916	0.86	0.40	0.91	5.42	0.08	0.39	0.21
	Avg	0.86	0.38	1.23	**5.04	0.06	**0.28	**0.18
	Std	0.07	0.03	0.96	0.70	0.01	0.06	0.04

Bold type indicates value is significantly different from control value
***ρ ≤ 0.05; **ρ ≤ 0.01**

Table L-6
Protocol No. 0E9V-30-11-05-01
Subacute Oral Toxicity of GDNP in Female Rats
Individual 14-Day Normalized Organ/Brain Weight Ratios

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
0	849	3.71	1.03	3.49	4.00	2.29	3.26
0	853	4.12	1.06	3.70	9.34	2.47	2.75
0	859	4.91	0.98	4.12	9.14	2.63	2.63
0	860	5.52	1.07	5.12	7.18	2.98	3.70
0	863	4.17	0.95	4.17	7.29	2.40	4.01
0	874	4.79	0.99	4.37	8.25	3.25	2.42
0	892	4.53	0.92	3.91	7.82	2.35	2.40
0	899	4.60	0.91	3.86	8.52	2.56	4.26
0	901	5.30	0.92	3.98	8.29	2.93	3.70
0	905	4.78	0.95	5.66	7.14	2.69	3.19
	Avg	4.64	0.98	4.24	7.70	2.65	3.23
	Std	0.55	0.06	0.67	1.51	0.31	0.67

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
1.3	852	4.07	0.89	4.24	7.41	3.07	1.85
1.3	857	5.56	0.93	3.56	9.36	3.04	2.63
1.3	867	4.39	1.06	4.20	7.60	2.34	3.80
1.3	870	4.16	0.84	3.92	8.65	2.27	2.38
1.3	883	4.31	0.93	3.88	7.73	2.32	3.09
1.3	894	5.18	1.09	4.13	7.69	2.82	2.10
1.3	896	4.75	1.03	4.02	7.18	2.87	2.32
1.3	897	4.49	1.02	4.14	9.18	2.70	2.30
1.3	900	5.16	1.04	4.36	9.47	3.16	1.79
1.3	907	5.35	1.03	4.17	9.09	1.98	1.93
	Avg	4.74	0.99	4.06	8.34	2.66	**2.42**
	Std	0.53	0.08	0.23	0.90	0.40	0.62

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
4.2	855	4.60	0.94	3.80	9.66	1.99	2.61
4.2	866	4.15	1.02	4.71	7.98	2.77	2.13
4.2	868	4.83	1.05	3.72	9.44	3.06	2.39
4.2	873	5.20	1.00	4.23	10.17	2.88	2.94
4.2	880	4.14	0.92	3.82	7.18	2.38	2.54
4.2	895	4.32	0.97	4.31	8.65	2.81	3.14
4.2	904	5.63	0.93	4.41	8.38	2.57	2.28
4.2	906	6.57	1.06	3.86	8.84	2.87	2.10
4.2	911	5.96	1.14	5.06	11.45	3.13	2.77
4.2	913	5.47	1.08	4.87	11.17	3.35	2.91
	Avg	5.09	1.01	4.28	**9.29**	2.78	**2.58**
	Std	0.82	0.07	0.48	1.36	0.39	0.36

Table L-6 continued

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
13.9	850	4.87	0.93	4.10	9.52	2.86	2.86
13.9	862	4.82	0.94	3.32	9.41	2.47	2.47
13.9	864	4.41	0.89	3.52	7.82	2.79	2.07
13.9	865	4.79	0.90	4.02	9.38	2.55	2.29
13.9	872	5.03	1.04	4.27	11.30	2.43	3.95
13.9	881	4.64	0.97	3.47	8.85	2.40	2.19
13.9	882	5.05	1.06	4.12	9.89	3.08	2.58
13.9	885	4.46	1.17	4.47	8.57	3.43	2.17
13.9	888	5.16	1.09	4.27	9.78	2.99	2.01
13.9	909	5.03	1.01	4.40	8.90	2.72	2.36
	Avg	4.83	1.00	4.00	**9.34**	2.77	**2.49**
	Std	0.26	0.09	0.41	0.93	0.33	0.57

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
45.9	851	5.37	0.91	3.71	7.43	2.34	3.09
45.9	858	4.46	0.86	4.18	10.29	2.23	4.51
45.9	869	4.03	0.97	3.92	9.68	2.53	3.44
45.9	871	4.14	0.89	3.89	7.47	1.95	2.47
45.9	875	3.96	0.82	3.84	7.61	2.49	2.28
45.9	876	3.91	0.82	3.80	8.94	2.07	3.13
45.9	878	4.08	0.91	4.52	7.65	2.30	1.94
45.9	887	5.32	0.59	4.38	9.14	2.85	1.99
45.9	889	4.69	0.98	4.80	10.17	2.60	2.60
45.9	903	4.18	0.91	4.32	9.41	2.35	4.00
	Avg	4.41	0.87	4.14	8.78	2.37	2.95
	Std	0.54	0.11	0.36	1.14	0.26	0.86

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
151.5	848	4.24	0.87	4.20	5.81	2.33	3.26
151.5	861	4.08	0.91	3.99	8.94	2.68	2.68
151.5	879	3.65	0.87	4.26	7.94	2.28	3.49
151.5	884	4.88	1.03	5.15	11.05	2.79	2.67
151.5	886	3.58	0.95	4.43	8.09	2.25	1.97
151.5	898	5.22	1.03	5.05	10.44	2.80	2.58
151.5	908	4.26	1.02	5.43	9.66	2.44	2.39
151.5	910	5.16	1.08	6.75	10.32	2.65	2.39
151.5	915	5.22	1.04	5.47	10.75	2.85	3.17
151.5	917	3.87	0.93	4.57	9.14	2.20	1.94
	Avg	4.42	0.97	4.93	**9.21**	2.53	**2.65**
	Std	0.65	0.08	0.83	1.61	0.25	0.52

Table L-6 continued

Dose mg/kg-day	Rat#	Heart	Kidney	Liver	Ovaries	Spleen	Uterus
500.0	854	4.33	0.98	4.44	6.43	2.81	2.28
500.0	856	4.36	0.98	5.37	6.91	2.39	1.91
500.0	877	3.68	1.05	5.03	5.75	2.53	1.15
500.0	890	4.55	1.18	6.36	8.56	4.22	2.51
500.0	891	4.24	4.57	7.10	5.81	3.09	1.40
500.0	893	4.78	1.18	6.07	7.07	3.42	2.39
500.0	902	4.51	1.08	5.94	8.67	3.58	2.20
500.0	912	4.54	1.13	6.30	8.65	2.65	1.73
500.0	914	4.86	1.14	6.08	8.67	3.58	2.83
500.0	916	4.69	1.07	6.33	8.94	4.58	2.40
	Avg	4.45	1.44	**5.90**	7.55	3.29	**2.08**
	Std	0.34	1.10	0.76	1.28	0.73	0.53

Bold type indicates value is significantly different from control value

Pathology Report for

0E9V-30-11-05-01

Effects of Acute Oral Guanidinium 3,4-dinitropyrazolate (GDNP) Exposure to Female Rats (*Rattus norvegicus*)

December 02, 2011

Prepared by:

Shannon M. Wallace, DVM, Diplomate, ACVP
LTC,VC

MCHB-IP-AU 13 May 2011

MEMORANDUM FOR Dr. Lawrence Williams

SUBJECT: Protocol Approval

1. USAPHC's (Prov) Institutional Animal Care and Use Committee (IACUC) has approved your protocol entitled, "Effects of Acute Oral Guanidinium 3,4-dinitropyrazolate (GDNP) Exposure to Female Rats (*Rattus norvegicus*)".

2. Your protocol has been assigned an IACUC approval number: **11 - 05 - 01**. Please refer to this number in all further correspondence regarding this protocol. The complete protocol study number is 0E9V-30-11-05-01.

3. Please report the total number of animals actually used and pain categories to the IACUC Administrator when your protocol is completed or at the end of the fiscal year, whichever is sooner. If you have not finished your protocol by the end of the fiscal year, report the animal numbers used through 30 September and their corresponding pain categories for the USDA report on the protocol annual review form. You will receive this annual form in October of each year the protocol is open. The annual review form requests additional information that is required for the DoD Annual Report to Congress on Animal Use.

4. The protocol should be followed exactly as you have described it to the IACUC. If you wish to make procedural changes, or require more animals than you anticipated, you must submit a protocol modification using CHPPM Form 28-R-E, being careful to include a justification for the change(s) to your original protocol. Refer to the most current versions of the USAPHC (prov) IACUC SOPs for more information concerning modifications.

KRISTIN T. NEWKIRK
IACUC Chair

ANIMAL USE PROTOCOL
PORTFOLIO OF TOXICOLOGY
ARMY INSTITUTE OF PUBLIC HEALTH
U.S. ARMY PUBLIC HEALTH COMMAND
ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: Effects of Acute Oral Guanidinium 3,4-dinitropyrazolate (GDNP) Exposure to Female Rats (*Rattus norvegicus*)

PROTOCOL NUMBER: ØE9V-3Ø-11-Ø5-Ø1

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SPONSOR: U.S. Army Research Development Engineering Command (RDECOM)
(AMSRD-MSF)
Environmental Acquisition and Logistics Sustainment Program
Aberdeen Proving Ground, MD 21010

I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the toxicity of a new experimental explosive, Guanidinium 3,4-dinitropyrazolate (GDNP), in the female rat after either a single dose, or short-term repetitive oral exposures. Rats are widely used as experimental subjects for toxicity studies and females will be used in this study as they are generally